Bisphenol-A and Risk of Obesity Among A Sample of Egyptian Children: Role of Adiponectin as Biomarker of Exposure

Hend M. Abo El-Atta¹, Adel M. El-Mansoury¹, Amany K. El-Hawary², Muhammad E.M. Abdel-Naby¹, Manar A. Helmy¹

ABSTRACT

Bisphenol-A (BPA) is one of the commonest chemicals used in the manufacturing of plastics. Childhood obesity is one of the most serious public health challenges of the 21st century that affects many low- and middle-income countries, including Egypt. This study was conducted to correlate between BPA exposure and risk of obesity in a sample of Egyptian children and to find out whether adiponectin (ADP) can be utilized as an exposure biomarker or not. This study was conducted on 80 children. They were divided into two groups according to their body mass index (BMI): Study group included 40 obese children (BMI ≥ 95th percentile), and control group included 40 normal-weight children (BMI 5th - 85th percentile). Levels of urinary BPA and serum ADP were estimated using HPLC and ELISA respectively. The study revealed that statistically significant increase in mean adjusted urinary BPA levels (P < 0.001) and serum ADP levels (P < 0.001) between study and control groups. Within the study group, a statistically significant positive correlation was found between urinary BPA levels and BMI (r = 0.956), meanwhile, statistically significant negative correlations were found between urinary BPA levels and serum ADP levels, as well, serum ADP levels and BMI (r= - 0.947, - 0.984 respectively). Binary logistic regression analysis showed that odds ratio for serum ADP level was 0.146 with 95% CI: 0.003-0.66, *P*=0.012. Significant increased risk for obesity among BPA exposed children was found and ADP can be used as a predictor biological marker for BPA-induced obesity.

Introduction

Bisphenol-A (BPA) is one of the commonest synthetic chemicals worldwide that is predominantly used in the manufacturing of polymers such as polycarbonate plastics and epoxy resins (Vom Saal et al., 2012). Besides, BPA is now well known as one of the endocrine disrupting chemicals that have the affinity to bind different hormonal receptors and either inhibit or enhance hormonal actions causing dysfunction to the endocrine and neuro-endocrine systems, and interfering with normal hormonal regulation (Anglea et al., 2013).

Worldwide, childhood obesity is one of the most serious public health challenges of the 21st century that steadily affects many low- and middle-income countries (Sibai et al., 2010). Children may be more susceptible to BPA exposure than adults as they still have immature organs, relatively low body weights and are going through a rapid physical development, thus, it could be a potential major public health concern, even with low levels of

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repeated exposure, in relation to the epidemic of childhood obesity (Rubin, 2011). Therefore, it is intriguing to hypothesize that exposure to BPA may have a role in weight gain and the development of obesity in Egyptian children.

Adiponectin is an insulin-sensitizing protein secreted from adipose tissue, and has been shown to improve glucose tolerance and insulin resistance in humans (Devasia et al., 2017). Many studies showed that metabolic syndrome, insulin resistance and obesity were associated with lower adiponectin (Pervanidou et al., 2013; Wilton et al., 2017). Menale et al. (2016) have declared an inverse association between adiponectin and BPA urinary levels independently in obese children; they explained that exposure to BPA leads to down-regulation of adiponectin expression.

This study aimed to correlate between bisphenol-A exposure and risk of obesity in a sample of Egyptian children, and to find out the role of adiponectin as a biomarker in bisphenol-A-induced childhood obesity.

**Subjects and Methods:**

**Subjects:**

A case-control study was conducted on 80 children 2-18 year-old of both sexes; recruited from Pediatric Endocrinology & Diabetes Unit, Mansoura University Children Hospital (MUCH), Egypt; during their primary visits. An approval from Mansoura University Institutional Research Board was taken and informed consents were obtained from children's mothers.

Anthropometric measurement (weight, height) and BMI were calculated to all the studied subjects according to Wang et al. (2014) and then they were divided into two groups. The first group "study group": 40 obese children (BMI ≥ 95th percentile). The second group "control group": 40 normal-weight children (BMI 5th - 85th percentile). Exclusion criteria: children with hepatic, renal, endocrine or genetic disorders, those with history of intra-uterine growth retardation, or corticosteroid therapy or born to diabetic mothers.

**Chemicals & Kits:**

HPLC chemicals: bisphenol-A (analytical standard ≥ 99.9 %), bisphenol-BP (BPBP, analytical standard ≥ 98 %), B-Glucuronidase enzyme (Type HP-2, from Helix Promatia, aqueous solution, activity ≥ 100,000 units/ml), acetonitrile, ethyl acetate, hydrochloric acid, sodium acetate buffer solution, potassium phosphate monobasic; were purchased from Sigma Aldrich Co., Egypt. ELISA kit Human adiponectin was purchased from SRB/Shanghai Sunred Biotechnology Comp., Ltd., Catalogue No. 201-12-1551.

**Methods**

Socio-demographic data including age, sex, residence and sources of exposure were obtained from all children's mothers.

From each child: 5 ml urine was collected and stored at -20 °C until analysis. In order to minimize external contamination with BPA during urine sampling and storage, the technicians were instructed to avoid contact of urine with plastic products during sample collection using glass centrifuge tubes (Wang et al., 2012). As well, 5 ml blood was collected into a glass centrifuge tube, left for serum coagulation at room temperature for 10-20 minutes, and then centrifuged for 15 min at the speed of 3500 r.p.m., and then the supernatant layer was aspirated. Finally serum samples were stored at -20 °C until analysis.

1. **Estimation of bisphenol-A using HPLC:**

   Extraction of BPA by enzymatic digestion was done through previously described method by Matsumoto et al. (2003). This was followed by estimating levels using reversed phase high performance liquid chromatography with florescence detector (Agilent technology 1260 series) at Forensic...
Medicine and Clinical Toxicology Department, Mansoura Faculty of Medicine, according to parameters mentioned in table (I). Calibration curve was performed by using five serial concentrations (10, 20, 60, 80 and 100 ng/ml).

After estimation of the total concentration of BPA (ng/ml) by the previously mentioned procedure, it was adjusted to urinary creatinine concentration (mg/dl) to correct the urine volume according to Wang et al. (2012).

2. Estimation of adiponectin using ELISA:

All blood samples were analyzed using ELISA-Reader Readwell Strip apparatus (Assay range: 02 - 60 mg/L, according to manufacture manual) at Clinical Pathology Department, Mansoura Faculty of Medicine.

Statistical Analysis

The collected data were processed and analyzed using the computer program SPSS (Statistical Package for Social Sciences) version release 20.0. Qualitative data were described using number and percent and quantitative data were described using median (minimum and maximum), mean and standard deviation. Student-t test and pearson correlation: for parametric quantitative variables. Mann Whitney test: for non-parametric quantitative variables. Binary logistic regression: for predictors detection. p-value is significant if < 5%

Results

Socio-demographic data:

Results and statistical analysis of socio-demographic data are shown in table (1).

Table (I): Chromatographic parameters used in the Agilent 1260 Infinity LC system.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Agilent 1260 Infinity LC system</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCC temperature:</td>
<td>40 °C.</td>
</tr>
<tr>
<td>FLD:</td>
<td>Ex: 230, Em: 316.</td>
</tr>
<tr>
<td>FLD acquisition rate, gain:</td>
<td>9.26 Hz, 15.</td>
</tr>
<tr>
<td>Mobile phase A:</td>
<td>10 mM monobasic potassium phosphate in water.</td>
</tr>
<tr>
<td>Mobile phase B:</td>
<td>100% Acetonitrile.</td>
</tr>
<tr>
<td>Gradient:</td>
<td>Time (min) %B</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>12.6</td>
</tr>
<tr>
<td></td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>18.1</td>
</tr>
<tr>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Flow: 0.9 ml/min.</td>
<td></td>
</tr>
<tr>
<td>Injection volume:</td>
<td>20 μL, 5 sec needle wash at flush port for 5 sec, using mobile phase A.</td>
</tr>
</tbody>
</table>

FLD: The fluorescence detector in liquid chromatography
The mean BMI was 33.32 ± 5.31 kg/m² in the study group, compared to 17.78 ± 1.11 kg/m² in the control group. There was statistically significant increase regarding the mean anthropometric values between both groups (p < 0.001).

Table (1): Statistical analysis of socio-demographic data of the studied subjects (n:80).

<table>
<thead>
<tr>
<th></th>
<th>Study group (n=40)</th>
<th>Control group (n=40)</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>11.38 ± 2.84</td>
<td>12.33 ± 2.03</td>
<td>t = 1.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p = 0.09</td>
</tr>
<tr>
<td>Range (min-max)</td>
<td>5.0 - 15.0</td>
<td>6.0 - 15.0</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>n (%)</td>
<td>n (%)</td>
<td>χ² = 1.53</td>
</tr>
<tr>
<td></td>
<td>31 (77.5)</td>
<td>26 (65.0)</td>
<td>p = 0.323</td>
</tr>
<tr>
<td>Girls</td>
<td>9 (22.5)</td>
<td>14 (35.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Residence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural</td>
<td>n (%)</td>
<td>n (%)</td>
<td>χ² = 0.952</td>
</tr>
<tr>
<td></td>
<td>30 (75.0)</td>
<td>26 (65.0)</td>
<td>p = 0.329</td>
</tr>
<tr>
<td>Urban</td>
<td>10 (25.0)</td>
<td>14 (35.0)</td>
<td></td>
</tr>
</tbody>
</table>

n: number, χ²: Chi square test, SD: standard deviation, t: Student-t test, min: minimum, max: maximum.

Figure (1) showed that all children in both groups (n = 80) were sharing the same dietary exposure sources, whether eating canned food or drinking water in plastic containers. As regard feeding during infancy (breast or plastic bottle feeding) or having dental filling, there were no statistically significant differences between the two groups (p = 0.823 and 0.592 respectively).

![Graph: Percentage of distribution of the studied groups according to their sources of exposure to bisphenol-A.](image)
Chromatographic results

By chromatographic analysis, the retention time of BPA standard was at 11.553 min. The curve was linear with correlation coefficient 0.98115, as shown in figure (2). The limit of detection (LOD) of the used method was 1.7 ng/ml. As regard the internal standard BPBP, its retention time was at 18.973 min (Figures 3 and 4).

The results of this study showed that serum ADP level can be used as a significant predictor for future obesity (odds ratio "OR": 0.146 with 95% confidence interval "CI": 0.003-0.66, p=0.012). On the other hand, as regard urinary BPA level, the statistical results could not establish the possibility that BPA could be a predictor for childhood obesity.

Fig. (2): Bisphenol-A standard calibration curve

Fig. (3): HPLC chromatogram of BPA in one obese child showing: (A) detectable urinary bisphenol-A level peak at retention time 11.153 minute (> LOD*), (B) bisphenol-BP internal standard peak at retention time 18.973 minute. *Limit of detection (LOD): 1.7 ng/ml.
Assay of urinary bisphenol-A and serum adiponectin levels in the studied children:

Statistical analysis of urinary bisphenol-A and serum adiponectin levels in the studied children is shown in table (2).

Table (2): Urinary bisphenol-A and serum adiponectin levels of the studied groups (n:80).

<table>
<thead>
<tr>
<th></th>
<th>Study group (n=40)</th>
<th>Control group (n=40)</th>
<th>Test of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary BPA level &quot;volume-based/unadjusted&quot; (ng/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (min-max)</td>
<td>46.72 (23.2 - 208.55)</td>
<td>7.58 (&lt; LOD - 31.3)</td>
<td>z = 6.9 p &lt; 0.001*</td>
</tr>
<tr>
<td>Urinary creatinine concentration (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40.75 (28.16 - 63.23)</td>
<td>45.32 (10.2 - 157.08)</td>
<td>z = 1.42 p = 0.157</td>
<td></td>
</tr>
<tr>
<td>Urinary BPA level &quot;creatinine-adjusted&quot; (μg BPA/g creatinine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (min-max)</td>
<td>121.89 (39.22 - 586.97)</td>
<td>14.92 (&lt; LOD - 34.94)</td>
<td>z = 6.98 p &lt; 0.001*</td>
</tr>
<tr>
<td>Serum ADP level (mg/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (min-max)</td>
<td>15.37 (6.42 - 21.02)</td>
<td>24.79 (19.53 - 126.75)</td>
<td>z = 7.61 p &lt; 0.001*</td>
</tr>
</tbody>
</table>

Figure (5) shows a statistically significant and strong positive correlation between creatinine-adjusted urinary BPA levels and BMI within the study group \( (p < 0.001) \), while in control group (Figure 6), a statistically significant and weak positive correlation among controls \( (p = 0.037) \) can be noticed.

**Fig. (5):** A diagram illustrating the correlation between creatinine-adjusted urinary bisphenol-A (BPA) levels and body mass index (BMI) in the study group \( (n = 40) \).

**Fig. (6):** A diagram illustrating the correlation between the creatinine-adjusted urinary bisphenol-A (BPA) levels and body mass index (BMI) in the control group \( (n = 40) \).
In figure (7), a statistically significant and strong negative correlation was detected between serum ADP levels and BMI in the study group (p < 0.001), while in the control group (Figure 8), a statistically significant and weak negative correlation (p = 0.017) was detected. In figure (9), a statistically significant and strong negative correlation between creatinine-adjusted urinary BPA levels and serum ADP levels in the study group (p < 0.001) was shown.

**Fig. (7):** A diagram illustrating the correlation between serum adiponectin (ADP) levels and body mass index (BMI) in the study group (n = 40).

**Fig. (8):** A diagram illustrating the correlation between serum adiponectin (ADP) levels and body mass index (BMI) in the control group (n = 40).
Fig. (9): A diagram illustrating the correlation between the creatinine-adjusted urinary bisphenol-A (BPA) levels and serum adiponectin (ADP) levels in the study group (n = 40).

Discussion

Obesity is one of the most important health hazards of BPA that has been extensively studied by many researchers worldwide especially in children (Ehrlich et al., 2016; Hao et al., 2017). In this study, authors evaluated the correlation between BPA exposure and risk of obesity in Egyptian children through estimating the degree of association between urinary BPA concentrations and children's body mass index outcomes in our locality, and to find out whether adiponectin can be utilized as a biomarker in BPA-induced obesity or not.

The statistical results showed that nearly half of the studied children were breast-fed during infancy, while others were fed in plastic baby bottles; as well for older children, who used to eat canned food or drink water in plastic containers. Owing to its properties, BPA can be easily released from the polymer products in which it is present during heating or exposure to acidic or alkaline medium. As a result, free BPA is released and migrates into food, beverages and environment, enhanced by repeated washing with detergents, rubbing and sterilization of the BPA-containing products, besides, it is well absorbed orally (EFSA, 2006).

In the current study, a statistically significant increase in average values of creatinine-adjusted urinary BPA levels was detected between the studied groups. Similar results were reported in a cross-sectional study conducted by Wang et al. (2014), concerning exposure to BPA among school children aged 9-12 years old in Eastern China. They found that in the high-BMI children, the creatinine-adjusted urinary BPA levels were higher compared to normal-BMI group.

As well, a statistically significant and direct correlation between the creatinine-adjusted urinary BPA levels and BMI was found in the study group. In agreement with these results, Wang et al. (2012) stated that daily BPA intake and urinary BPA concentration; were positively associated with BMI in Chinese school children. Similarly, Bhandari et al. (2013)
and Li et al. (2013) observed that higher urinary BPA level was associated with overweight and obese children. However, studies conducted to prove such correlation are still sparse and inconsistent. Another study that was conducted by Li et al. (2017) on American children declared that higher BPA levels may be associated with elevated lean body mass in boys, while in girls, higher BPA levels may be associated with elevated fat mass.

This strong positive correlation between urinary BPA levels and BMI was explained by several in-vitro and in vivo studies: in in-vitro studies, it was accounted to its ability to trigger the differentiation of fibroblasts into adipocytes, as well, stimulating and accelerating the adipocyte conversion process, besides, enhancing basal and insulin-stimulated glucose uptake and lipid accumulation in adipocytes (Angla et al., 2013). Confirming the results from in-vitro studies, the conducted in-vivo animal studies showed similar results to support the in-vitro studies' findings (Miyawaki et al., 2007; Patisaul and Bateman, 2008). That was explained with the ability of BPA to disrupt pancreatic β-cell function (Ropero et al., 2008) or interaction with ER-β (Soriano et al., 2012); and subsequently results in insulin resistance. On the other hand, a study was conducted by Ishido et al. (2004), they reported either no significant or negative association between BPA and body weight.

Regarding serum ADP levels, a statistically significant reduction was found. Similarly, Diamond et al. (2004) found that mean serum ADP level in obese children was 9.1 ± 3.7 mg/L, compared to non-obese children (17.1 ± 12.3 mg/L). As well, Panagopoulou et al. (2008) found that serum ADP levels were significantly lower in obese than non-obese children (8.86 ± 3.86 versus 13.08 ± 5.48 mg/L respectively).

In the current work, a statistically significant and negative correlation was found between the serum ADP levels and BMI in the obese children. This finding goes in accordance with Panagopoulou et al. (2008) who reported a significant negative correlation between decreasing serum ADP levels and increasing BMI among Greek children. Similar data were declared from previous similar studies conducted on people of different ethnic groups, such as Mexicans (Cruz et al., 2004); African Americans (Bush et al., 2005) and Asian (Tsou et al., 2004; Ogawa et al., 2005). On contrary, a German study that was conducted by Reinehr et al. (2004) found that ADP was negatively correlated only to percentage of body fat and no significant correlation between ADP and SDS-BMI.

To the best of our knowledge, very limited previously reported studies assessing the correlation between urinary BPA levels and serum ADP levels among human (Rönn et al., 2014), they found that BPA is associated positively with the adiponectin release in human. However, several in-vitro studies have investigated the effect of BPA, as an environmental pollutant, on the kinetic pathways of the adipose tissue metabolic hormones, including ADP (Kidani et al., 2010; Menale et al., 2016). They declared negative correlation between exposure to BPA and expression of ADP. The present study is considered the first Egyptian study that investigated the correlation between BPA and ADP in obese children.

In the current study, serum ADP level was found to be a significant predictor for childhood obesity, meanwhile, BPA could not be established as a possible predictor, a result that differ from those of Bhandari et al. (2013) and Li et al. (2013), who reported a dose-response
relationship between increasing urinary BPA concentrations and increased risk for future overweight and obesity. This observed contrast between the current results and those reported in the previously mentioned studies regarding BPA prediction for obesity can be explained by relatively much smaller section of population in this study that is considered a limitation to the results.

**Conclusion and recommendation**

The finding of this study proved that children with high BMI had significantly higher urinary BPA levels and lower serum ADP levels than those with normal BMI. This indicates significant associations between increasing urinary BPA levels, decreasing serum ADP levels and increasing BMI in children, with the possibility that ADP can be used as a biomarker for BPA-induced obesity.

Since children are more vulnerable to BPA exposure than adults, BPA may represent a novel, modifiable risk factor in relation to the epidemic of childhood obesity. Accordingly, further investigations are warranted regarding the biological effects of BPA on adipose tissue; as well as on the kinetics of different biomarkers that may have a role in induction of childhood obesity.

**Acknowledgment**

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El-Atta et al.


البيسفينول (أ) وخطر السمنة بين عينة من الأطفال المصريين: دور الأدينوبينكتين

كعلامة بيولوجية للتعرض

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د. محمد العدل، د. منار عادل حلمى
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يعتبر البيسفينول (أ) أحد أكثر المواد الكيميائية شيوعاً في تصنيع اللذان. وتعتبر البذلة في مرحلة الطفولة من أخطر تحديات الصحة العامة في القرن الحادي والعشرين والتي تؤثر على العديد من البلدان ذات الدخل المنخفض والموسط، بما في ذلك مصر. أجريت هذه الدراسة لمعرفة العلاقة بين التعرض للبيسفينول (أ) وخطر السمنة في عينة من الأطفال المصريين ومعرفة ما إذا كان يمكن استخدام الأدينوبينكتين كعلامة بيولوجية للتعرض أم لا. أجريت هذه الدراسة على 80 طفل. وقد تم تقسيمهم إلى مجموعتين وفقاً لمؤشر كتلة الجسم: المجموعة الدراسة ضمت 40 طفل يعانون من السمنة المفرطة (مؤشر كتلة الجسم ≥ 95٪)، والمجموعة الضابطة ضمت 40 طفل من ذوي الوزن الطبيعي (مؤشر كتلة الجسم 5 - 85٪). وقد تم تقسيم مسارات البيسفينول (أ) في البول ومستويات الأدينوبينكتين في البول باستخدام تقنية السائل اللوني عالي الأداء وتقنية الفحص المناعي الإلزامي على التوالي، في جميع الأطفال. وكشفت الدراسة عن وجود زيادة ذات دلالة إحصائية في متوسط مستويات البيسفينول (أ) المعدلة في البول (0.01<P<0.001) ومستويات الأدينوبينكتين في مصل الدم (P<0.001) بين مجموعات الدراسة والمجموعة الضابطة. كما أظهرت الدراسة وجود علاقة إرتباط معينة ذات دلالة إحصائية بين مستويات البيسفينول (أ) البولية ومترشح كتلة الجسم (0.956 = 2)، وجدت أسباب سلبية ذات دلالة إحصائية بين مستويات البيسفينول (أ) البولية ومستويات الأدينوبينكتين في مصل الدم، وكذلك مستويات الأدينوبينكتين في الدم ومؤشر كتلة الجسم (0.947 = 3، 0.984 - 0.04 على التوالي). كما أظهر تحليل الانحدار اللوجستي الثنائي أن نسبة الأرجحية لمستوى الأدينوبينكتين في الدم كانت 14٪ (0.0006-0.001). ومن هذه النتائج نستخلص حدوث زيادة كبيرة في خطر السمنة بين الأطفال المعرضين للبيسفينول (أ) ويمكن استخدام الأدينوبينكتين كعلامة بيولوجية لتوقع البدانة الناجم عن البيسفينول (أ).