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Bisphenol A in Children with Bronchial Asthma and Its Relation to Body Mass Index

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ABSTRACT

Background: One of the most debilitating diseases in children is bronchial asthma. Little research has been conducted into the relationship between bisphenol A (BPA), a commonly recycled monomer, which is linked to childhood obesity and asthma. **Objective:** This study's aimed to examine the relationships between paediatric obesity morbidity and bisphenol A (BPA).

Subjects and Methods:100 kids between 4 and 10 years were included in this study (25 asthmatics, 25 asthmatics with obesity, 25 obese non-asthmatics, and 25 healthy controls). The diagnosis of asthmatic children was made according to GINA guidelines (Global Initiative for Asthma). The BPA competitive ELISA Kit was used to measure the BPA levels in urine specimens. Looked up for the effect of BPA as a predictor of asthma development in asthmatic children. Additionally, research was done on BPA's potential role in the prediction of childhood obesity.

Results: High median total urinary BPA levels ($p > 0.001$) detected significantly in children with asthma and obesity. Increased levels of BPA were found to be a significant predictor ($p = 0.006$) in an analysis using multiple logistic regression of asthma predictors.

Conclusions: It's possible that BPA played a role in the development of bronchial asthma & obesity, based on the correlation between high BPA levels in urine with asthma diagnostic assessments and children obesity. There is a need for more clinical and biochemical study**.**

Keywords: Asthma, Bisphenol A, Obesity, ELISA.

INTRODUCTION

Children and adults alike can suffer from asthma, as chronic condition. Inflammation and muscle contraction around the tiny airways cause the airways in the respiratory system to narrow more. Coughing, wheezing, breathing difficulties, and tightness in the chest are all indications of asthma. These feelings are severe at night or right after exercise. Dust, smoke, fumes, temperature changes, animal fur, strong detergent, and perfume can all cause asthmatic attacks. Virus infections (colds) can also cause exacerbations [1].

Significant increase was noticed in the occurrence of childhood obesity within recent years, which is unequally distributed across countries and populations, posing an important public health problem with numerous health and social consequences. Obese children have a greater than 50% chance of developing obesity-related diseases as adults, including type 2 diabetes, dyslipidemia, and hypertension[2].Children's obesity has become one of the most serious public health challenges in the twenty-first century, negatively impacting all countries. Overweight has more than tenfold increased among school-aged children and adolescents in recent decades[3].The terms obesity and overweight refer to abnormal or excess fat accumulations that may be harmful to a person's health. A measurement used to assess childhood obesity and overweight is BMI. For children and adolescents in the same age and sex, overweight is defined as body mass index (BMI)within or over the eighty fifth percentile but less than the ninety fifth percentile. A body mass index (BMI) of 95th percentile or higher is considered obese in kids and teenagers of the same sex and age [4].

Among the most prevalent emerging pollutants in the environment is bisphenol A (BPA). It was first used as a monomer in the manufacturing of plastics, which were subjected to make toys, eyeglasses, computers, kitchen appliances, medical equipments, food and beverage metal coating containers, and other items. Epoxy resins with chemicals in them are used to make metal products like office, dairy, and dental equipment. BPA has also been discovered in human saliva, milk and urine[6]. By causing eosinophilic airway inflammation and corticosteroid insensitivity, Inhalation of BPA has been interconnected to oxidative stress, which has been linked to asthma pathogenicity, as well as poor asthmatic control and acute exacerbations[7].

BPA functions as an endocrine disruptor because of its ability to bind to the oestrogen receptors (ER) and fit into its binding pocket. BPA interacts with the thyroid receptors and androgen receptors (ARs) at higher concentrations [8].

This study aimed to look into any possible link that bind the urinary BPA levels and children's vulnerability for obesity and bronchial asthma.

SUBJECTS & METHOD

Study design

Our study was case control one conducted over 6 months from September 2020 to April 2021including:

1. Twenty-five children diagnosed to be obese non asthmatic (exogenous obesity) following up in Diabetes clinic of Endocrine & Metabolism Pediatric Unit (DEMPU), Cairo University.
2. Twenty-five children diagnosed to be asthmatic following up in asthma clinic of pulmonology Pediatric unite, Cairo University.
3. Twenty-five children diagnosed to be asthmatic

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with obesity following up in asthma clinic of pulmonology Pediatric unite, Cairo University.

4. Twenty-five children healthy, non-overweight &non obese children (control) will be recruited from general clinic.

Inclusion criteria: Asthmatic patients, healthy children and obese children, Childrenaged4to10 years and of both sexes.

Exclusion criteria: History of congenital heart disease. Anomalies causing narrowing of the airway. Repeated lower respiratory infection. Febrile children having chest infection.

Sample size:

There are 100 patients in the study who met the inclusion criteria. Four groups of 25 patients. Using G*Power software for sample size calculation[9]. Primary outcome measure is the relationship between asthma or obesity and serum bisphenol A levels, as well as their interaction. Using two-way ANOVA with two factors (main effects), we will get four possible groups (2 levels for factor A * 2 levels for factor B) and an interaction (numerator) degree of freedom of one ([2 - 1] * [2 - 1]).So, it is calculated that a sample size of 25 patients per group (total 100 patients) achieves 80% power to detect a medium effect size (Cohen*f*) of0.28 as regards bisphenol A level assuming a numerator degree of freedom of 1 and a confidence level of 95% (alpha error of 0.05).

Ethical approval:

Ethical committee of Kasr Al-Ainy, Faculty of Medicine approved the used procedures and written consent was obtained from parent of each child after the protocol of work was fully explained. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

All participants were subjected to the following:

- Careful Family history of asthma or obesity and Past history of exposure to any products contain BPA were taken.
- Careful clinical examination including symptoms of respiratory distress, whizzes, dyspnea, weight, height, hip and waist circumference measurements.

Urinary BPA measurement:

The urinary concentration was determined using the Bisphenol A (BPA) competitive ELISA Kit (Cell Biolabs, Inc. San Diego, CAEDTA, USA) The microplate reader: a Stat Fax 2100 microplate reader (Awareness Technology Inc. Palm City, FL, USA) determined the optical density (OD value) of each well.

Specimen collection and preparation:

Sterile tubes were used to collect the children urine then a centrifuge of 2000-3000 R per minute was used for 20 minutes. Then samples were reserved at -20 Celsius before analysis. Avoid repeat thawing and freezing.

Test principle:

The plate of wells coated with human BPA antibody. The sample containing BPA binds to the wells coated with antibodies. Human BPA antibody, which was biotinylated, added to the sample will bind to the BPA. During the washing step of the incubated plate, the Streptavidin-HRP that was unbound would be washed away. After that, by adding the substrate solution, and the developed color would be proportional to the human BPA amount. Stopping the reaction on adding solution of acidic ph, the measuring wavelength absorbance is 450 nm [10].

Result Calculation:

The value of the used standard optical density plotted on (Y) axis and its concentration on (X) axis horizontally to make standard curve and then create a best-fit curve through the graph's points. Computer-based curve-fitting software carried out these calculations, the best fit line was determined using regression analysis.

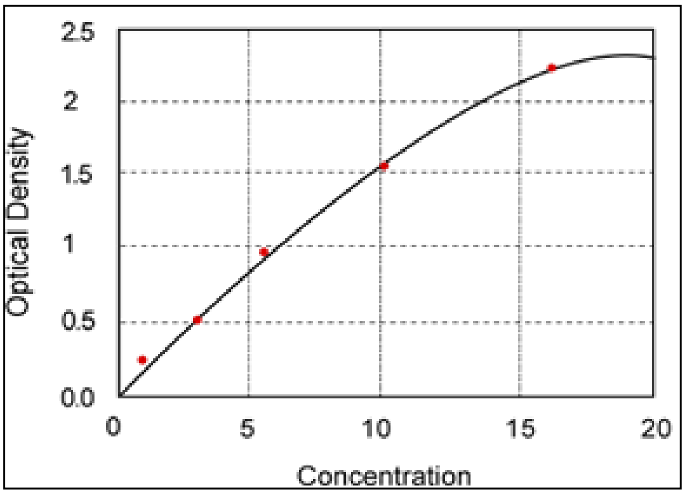

Figure (1): standard curve Bisphenol A (BPA)

Statistical methods

The statistical package for the social sciences (SPSS) (IBM Corp., Armonk, NY, USA) used for quantitative values, standard deviation, mean, and median. Frequencies and percentages used for categorical variables. For comparisons of normally distributed quantitative variables unpaired t test or ANOVA analysis were used, for the multiple comparisons post hoc test was used [11]. When the expected frequency is less than 5, Chi square test was used to compare categorized data [12]. Correlations between quantitative variables were calculated by Pearson correlation coefficient. To identify independent predictors of BPA, linear regression analysis was used. Logistic regression was used to identify independent asthma predictors. Results with P-values equal or less than 0.05 was defined as Statistical significance.

RESULTS

We perform our study on 100 children aged from 4 to 10 years and divided to four groups 25 case bronchial asthma group, 25 case bronchial asthma with obesity group, 25 case obese non asthmatic group and 25 control group. Table 1 and Figure 2 show that asthmatic patients (asthma group and asthma with obesity group) differ significantly from non-asthmatic patients (obese non asthmatic and controls groups). Asthmatic patients had significantly higher Urinary BPA levels than non-asthmatic patients (p value >0.001). There were non-significant differences in the other metrics (BMI, waist: hip and others) as shown in table (1) and figure (2).

Table (1): Comparison between asthmatic patients and non- asthmatic patients regarding anthropometric measure and BPA level

	Asthmatic (NO=50)	Non-asthmatic (NO=50)	
	Mean± SD	Mean± SD	P value
Weight(kg)	28.73±8.88	31.51± 10.38	0.194
Weight (SDS)	1.54± 0.14	1.74± 0.06	0.370
Height (cm)	117.72±12.92	122.70±13.07	0.058
Height (SDS)	0.40± 0.07	0.79 ± 0.27	0.061
BMI (kg/m2)	20.10± 3.79	20.46±3.68	0.631
BMI (SDS)	2.13± 0.37	2.16± 1.02	0.699
Waist Circumference(cm)	63.58± 9.22	65.44± 11.49	0.372
Hip Circumference(cm)	66.94± 7.53	70.09± 11.13	0.101
Waist: hip	0.94± 0.05	0.93± 0.07	0.253
Waist: height	0.54± 0.06	0.53± 0.07	0.527
Urinary BPA level(ng/ml)	40.69 ±11.40	32.05± 11.19	< 0.001

Unpaired t-test BPA=bisphenol A

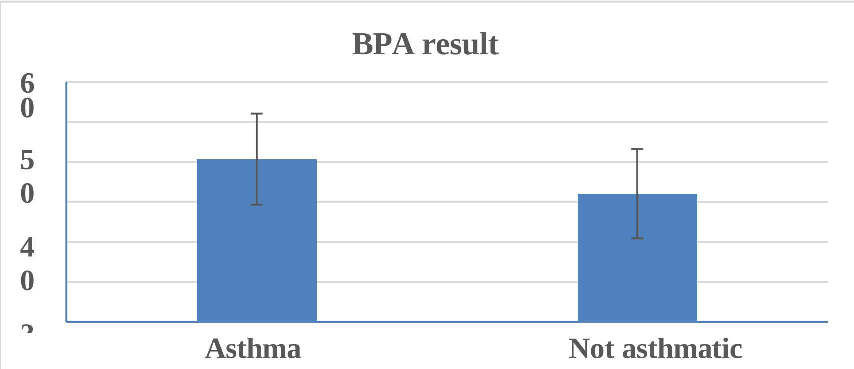

Figure (2): Urine BPA level in asthmatic cand non-asthmatic groups(p-value>0.001)

Table (2) and figure (3) showed that there was a significant difference between obese and non-obese patients (obese group and asthma with obesity group) (asthmatic and controls groups). Obese patients had significantly higher urinary BPA levels than non-asthmatic patients. Weight, BMI, SDS, waist measurement, hip size, and waist: hip ratio, or Waist: height ratio showed significant differences. However, there was no discernible difference in terms of height.

Table (2): Comparison between obese patients and non-obese patients regarding anthropometric measurement and BPA level

	Obese NO=50)	Non-Obese (NO=50)	
	Mean± SD	Mean± SD	P-value
Wt (k)	33.94±11.48	26.30±8.30	<0.001
Wt (SDS)	2.53±0.63	0.74±0.65	<0.001
Ht (cm)	119.42±13.44	121.00±12.98	0.551
Ht (SDS)	0.95 ±0.15	0.54 ±0.06	0.975
BM index (kg/m2)	23.06±2.67	17.50±2.27	<0.001
BM index (SDS)	3.34 ±0.49	0.94±0.85	<0.001
Circumference of Waist(cm)	70.71±10.26	58.31±5.91	<0.001
Circumference of Hip(cm)	71.17±10.16	65.85±8.24	0.005
waist: hip	0.99 ±0.02	0.88 ±0.03	<0.001
waist: height	0.59 ±0.04	0.48 ±0.02	<0.001
Level of urinary BPA (ng/ml)	40.35±11.38	32.40±11.47	0.001

Unpaired t test BMI=body mass index BPA=bisphenol A

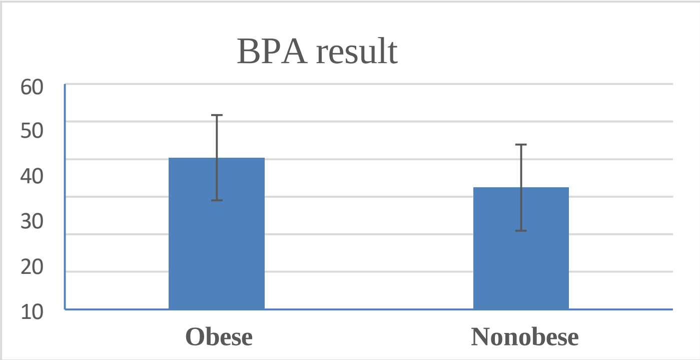

Figure (3): Urine BPA level in obese and non-obese groups(p-value=0.001).

Table (3) and figure (4) showed a significant difference in BPA level between cases and control group. Obese and asthmatic children recorded higher levels of BPA than control group.

Table (3): Comparison between four groups regarding BPA level

	Asthma group (No=25)	**Asthma with obesity group (No=25)**	**Obese Non-asthmatic group (No=25)**	**Control group (No=25)**	
	Mean± SD	**Mean± SD**	**Mean± SD**	**Mean± SD**	**P value**
Urine BPA level (ng/ml)	39.19±1.10	42.20± 10.69	38.50± 1.96	25.61± 4.09	<0.001

BPA=bisphenol A

Table (4) and figure 4 show significant positive correlation between urine BPA level and weight SDS (r=0.257, p=0.010), BMI (r=0.249, p=0.012), BMI SDS (r=0.313, p=0.001), waist to hip ratio (r=0.342, p>0.001) and waist to height ratio (r=0.263, p=0.008). but no significant correlation between urinary BPA level with age, weight, height, height SDS, WC and HC.

Table (4): Comparison between each2 groups in BPA level

	Asthma group .(NO=25) vs Asthma with Obesity group (NO=25)	**Asthma Group (NO=25) vs Obese group (NO=25)**	**Asthma group (NO=25) vs Control group (NO=25)**	**Asthma with obesity group (NO=25) vs Obese non-asthmatic group (NO=25)**	**Asthma with obesity group (NO=25) vs Control group (NO=25)**	**Obese non asthmatic group (NO=25) vs Control group (NO=25)**
Urinary BPA level (ng/ml)	1.000	1.000	<0.001	1.000	<0.001	<0.001

BPA=bisphenol A VS= versus

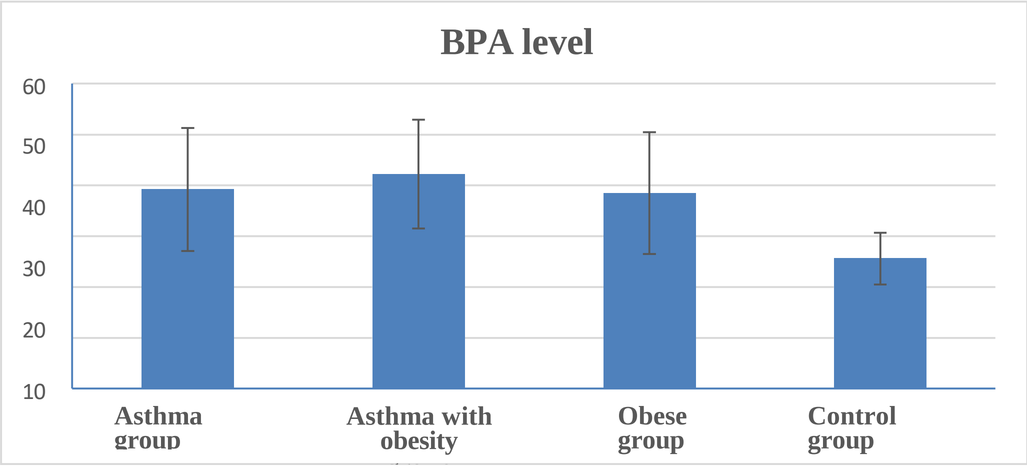

Figure (4): BP- A level in the four groups (p-value>0.001)

There was significant positive correlation between urine BPA level and weight SDS (r=0.257, p=0.010), BMI (r=0.249, p=0.012), BMISDS (r=0.313, p=0.001), waist to hip ratio (r=0.342, p>0.001) and waist to height ratio (r=0.263, p=0.008). But there was no significant correlation between urinary BPA level with age, weight, height, height SDS, WC and HC as shown in table (5) and figures (5, 6, 7, 8 and 9).

Table (5): Correlation between bisphenol A and all the clinical and lab data in the whole study group

	BPA level(ng/ml) (NO=100)	
	r	P-value
Age(years)	-0.079-	0.437
Weight(kg)	0.078	0.441
Weight SD	0.257	0.010
Height(cm)	-0.090-	0.373
Weight SD	-0.066-	0.516
BM Index kg/m2	0.249	0.012
BM Index SD	0.313	0.001
Circumference of Waist	0.135	0.181
Circumference of Hip	-0.008-	0.938
Waist: hip	0.342	<0.001
Waist: height	0.263	0.008

Pearson Correlation **BPA=bisphenol A BMI=body mass index**

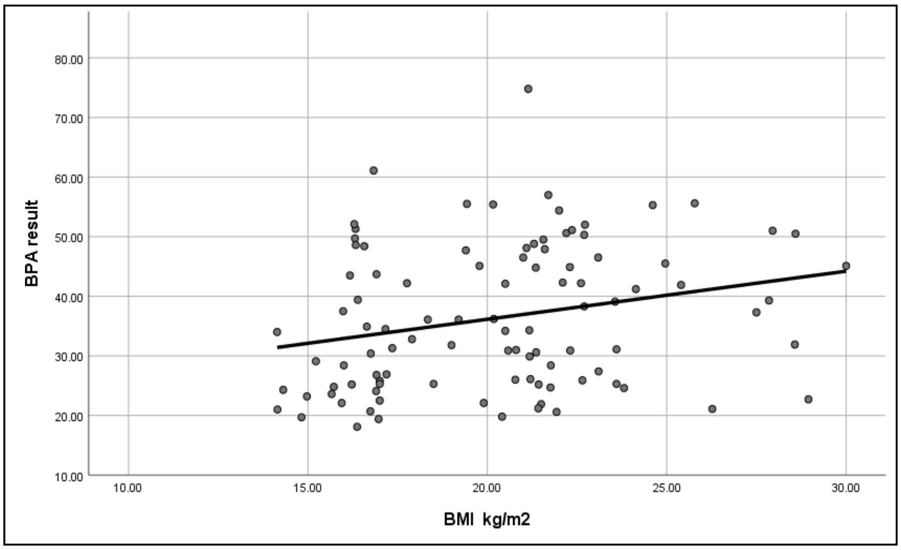

Figure (5): Correlation between urine BPA level and BMI (r=0.249, p=0.012)

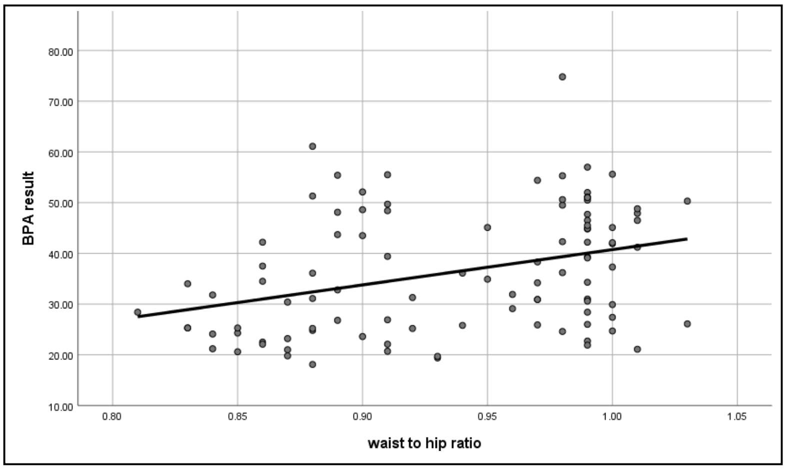

Figure (6): Correlation between urine BPA level& ratio of waist to hips (r=0.342, p>0.001)

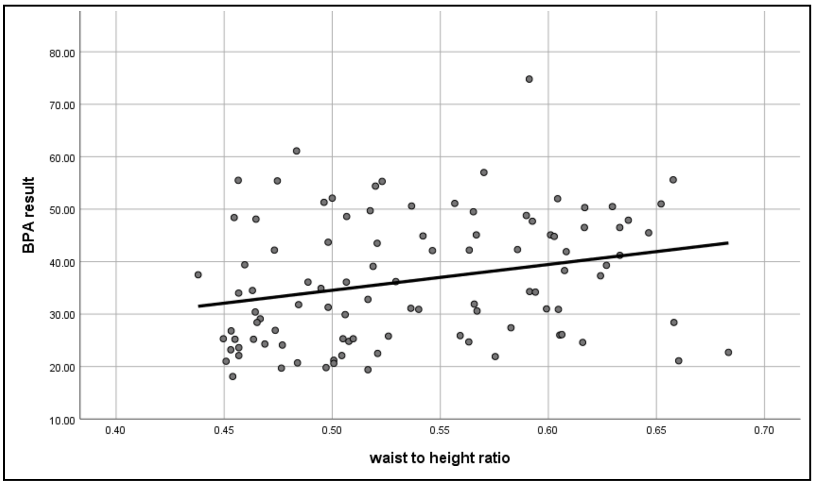

Figure (7): Correlation between urine BPA level& waist to height ratio (r= 0.263, p=0.008)

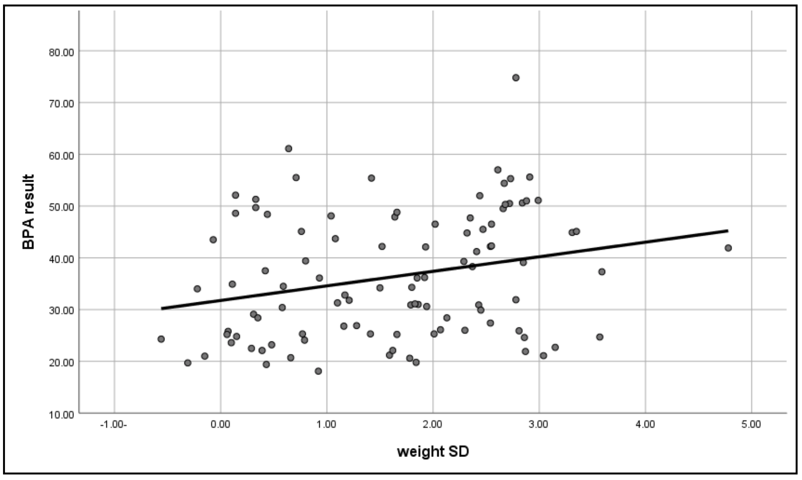

Figure (8): Correlation between weight SD &urine BPA level (r=0.0.257, p=0.010)

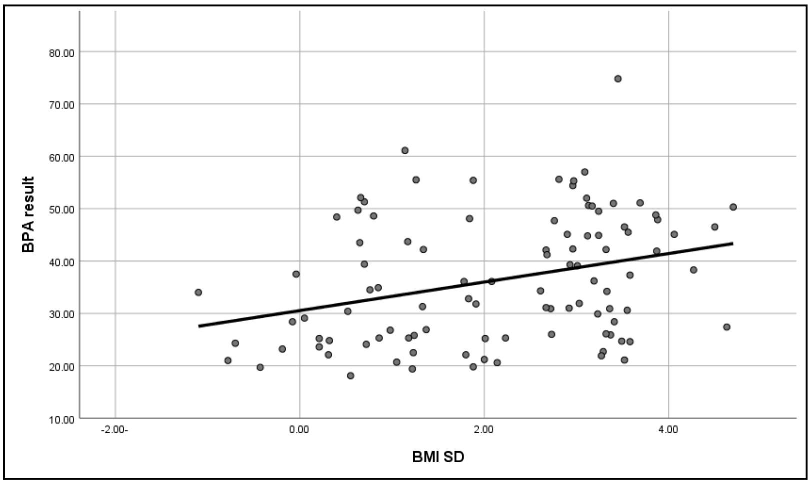

Figure (9): Correlation between urine BPA level and BMISD. (r=0.313, p=0.001).

Table (6) showed that waist to hip ratio was a good predictor for BPA level in obese children.

Table (6): To identify the obesity indices' predictive value for BPA using linear regression analysis

Model		Unstandardized Coefficients		Standardized Coefficients	t	P-value	95.0%Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
Urine BPA (ng/ml)	(Constant)	-28.878-	18.145		-1.592-	0.115	-64.885-	7.130
	Waist to hip ratio	69.623	19.322	0.342	3.603	< 0.001	31.278	107.968

BPA=bisphenol A

Table (7) showed that the level of Urine BPA and positive family history of asthma was good predictors of asthma.

Table (7): Analysis of multiple logistic regressions to predict asthma

		P-value	OR	95%C.I.	
				Lower	Upper
Asthma	Family history of asthma	0.006	3.462	1.430	8.383
	Urine BPA level (ng/ml)	0.006	1.058	1.016	1.101

BPA=bisphenol A

DISCUSSION

One of the commonest chronic diseases children are suffering from is asthma. It is a disease resulting in chronic inflammatory effect of respiratory airway with recurrent episodes of airflow obstruction by the effect of edema, bronchospasm, and increased mucus production. Asthma is commonly associated with allergic rhinitis and eczema, and called the atopic triad. Asthmatic patients may experience a variable symptoms of respiratory system affection like wheezes, breath shortness, chest tightness and coughing. The symptoms frequency and severity varied, but the deadly consequences of the uncontrolled asthma are the respiratory failure and death [13]. Asthma is largely caused by the environment and has multiple causes. Asthma is extremely common, particularly in impoverished urban areas. Asthma is the third leading cause of paediatric hospitalization [14].

On national and international scale, the childhood obesity is known to be significant community health issue. Over last few years, the prevalence of childhood obesity has risen. It is caused by a discrepancy between calorie intake and calorie expenditure [15]. Obesity in childhood has consequences for health during childhood and later in adult life. Not only numerous acute health problems caused by obesity but also great suffering from type 2 diabetes, menstrual irregularities, early puberty and hypertension and psychological problems related to increased children BMI [16].

BPA is highly used chemical that is commonly used to line the inside of food cans with polycarbonate plastics and epoxy resins. Although BPA is no longer permitted in some countries for manufacturing feeding bottles of infant, it is still used as plastics polycarbonate monomer used in production of toys, feeding utensils, pacifiers, and teething rings [17]. Evidence over the last two decades that PA has been shown to be an endocrine disruptor that disturb a variable hormonal physiological pathway. In addition to research linking BPA exposure to negative effects on children's health, including asthma and obesity [17].

Our study aimed to detect the BPA level in urine of both, obese and asthmatic children, and to evaluate its potential relation to obesity and asthma.

In the current study, 100 children aged 4 to 10 were included. 25 obese children (exogenous obesity) were followed up at Cairo University's Diabetes, Endocrine and Metabolism Pediatric Unit (DEMPU). In the pulmonology Pediatric Unit at the Asthma Clinic of Cairo University, 50 children were diagnosed with bronchial asthma (25 asthmatic non obese and 25 asthmatics with obesity). In comparison to a control group of 25 apparently healthy non-obese or asthmatic children of matched age & gender. All subjects provided a detailed medical and family history. At the time of enrollment, all children were subjected to a physical examination, which included a chest examination (inspection, auscultation), weight, standing height, BMI calculation, circumference of waist and hip, waist : hip, and waist : height and urinary BPA levels were analyzed.

Levels of BPA in urinary sample were significantly high in asthmatic children group compared to the healthy control (39.19 ng/ml in children with asthma vs 25.61ng/ml in control group, p>0.001) and (42.20ng/ml in asthmatic with asthma vs 25.61ng/ml in control group, p >0001), high BPA levels are important as a significant asthma predictor according to multiple logistic regression analysis (p= 0.006, OR 1.058, 95%CI 1.016-1.101).Such an outcome is in line with the findings of **Youssef *et al*.**[18]where 97 subjects (52 healthy controls and 45 asthmatic child) aged 3-8 years. They used to choose asthmatic children according to the guidelines of Global initiative for asthma (GINA). Urinary levels of BPA were found to be significantly greater in asthma patients than in healthy group.

The children with urinary BPA level greater than 1.3 ng/ml was susceptible to asthma. Child with BPA level>1.3 ng/mL had stronger indicator for asthma occurrence than being indicator for exposure to passive smoking (PS). This was revealed by multiple logistic regression analysis (p=0.006 for BPA vs. p=0.049 for PS). Our results are in line with findings of **Kim *et al*.**[19], their study involved 127 children, 7-8 years old ofage without a history of asthma diagnosis in a Korean school. Three surveys were carried, each 2 years apart. The creatinine-adjusted urinary bisphenol was log-transformed. At ages 11-12 years, wheezing was associated with certain BPA concentration (P=0.02) as well as asthma occurrence(P=0.001). **Quirós-Alcalá *et al*.**[7]assessed BPA concentrations in urine specimens from 148 child (5-17 years old) with existing asthma. The study discovered a link between BPA exposure and asthma morbidity metrics. Ten times increase in BPA concentrations would increase the chances of general severe symptoms occurrence, and emergency department intention.

Our results are in line with those of **Amin *et al*.**[20], who investigated the relation between BPA exposure and obesity in 132 children &young adult(6 - 18 years old)from Isfahan, Iran. The results clarified significant increase in the mean waist circumference and BMI across the BPA tertiles(p=0.001). The same findings were observed regarding fasting blood sugar, systolic and diastolic blood pressure. The third tertile of BPA participants had an obesity risk that was 12.48 times greater than that of the other participants (95% CI: 3.36-46.39, p 0.001). Also, **Trasande *et al***.[21]analysis of 2838 participants (6 - 19 years old) who were randomly selected for measuring the urinary BPA concentration between(2003-2008).Analysis revealed significant increase in BPA urinary levels that was associated with obesity of children &young adult.

Our results are like those of **Li *et al***. [22] who studied students from grades 4 to 12 indifferent Shanghai schools. The study found that an elevated urine BPA level (2 g/L) was coupled to more than two fold increase in the risk of being overweight, the level was corresponding to the median urine BPA level in the US population. The study found also a dose-depend relationship between increasing urine BPA levels and an increased risk of being overweight (p=0.006 for trend test). ***Wang et al.*** [23] performed an investigation study for the correlation between the exposure of school children to BPA and their body mass index (BMI). In 84.9%of urine specimens analyzed the BPA was with 0.45 ng/mL concentration with range of (0.03g/day-1.96g/day) estimated daily intake, and quantitative mean of 0.37 g/day. Estimated daily intake and mean urinary BPA concentrations were compared for boys and girls, but it was significantly higher among older children than younger children. Multiple linear regression analysis revealed a significant association between urinary BPA concentrations and increasing BMI values in all subjects after age and gender modification, and the results were comparable before and after specific gravity of urine was adjusted. Before the specific gravity corrected, significant associations were in females and those 8-11 years old when categorised by age or sex. Association between BMI and daily intake estimates were also found.

CONCLUSION

- Higher levels of BPA in the urine have been associated with the diagnosis of asthma in children, suggesting that BPA exposure may have a role in the development of bronchial asthma.
- Increase BPA level may worth the asthma control.
- This study clarified the possible association between BPA exposure and childhood obesity.
- BPA should be considered an obesogenic environmental compound.

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