
CORRELATION BETWEEN URINARY BISPHENOL A LEVELS WITH INSULIN RESISTANCE AND DIABETES COMPLICATIONS IN CHILDREN WITH TYPE 1 DIABETES MELLITUS

By

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ABSTRACT

Introduction: Bisphenol A (BPA) is ubiquitous in our environment, as evidenced by the fact that over 90 % of individuals have detectable levels of BPA present in their urine, which is the primary route of excretion in humans. BPA has been found in neonates, children and adults, and can be measured in a range of body fluids and tissues, including urine, blood, saliva, placental tissue, adipose tissue and breast milk (Vandenberg et al., 2010).

Objectives: The aim of this study was to assess the association between BPA exposures by measuring urinary levels of BPA, insulin resistance using estimated glucose disposal rate, glycemic control using mean value of HbA1C and diabetes complications in type 1 diabetic children.

Subjects and Methods: This is a descriptive cross-sectional study that was carried out on 100 children with type 1 DM. they are selected by simple random method from the attendants of Pediatric Endocrinology Outpatient Clinic, Abo Elrish Pediatric Hospital, Cairo University, Egypt and Pediatric Endocrinology Outpatient Clinic of EL-Hussein University Hospital, Al Azhar University during the period from April 2019 to March 2021.

Results: The mean age of the studied diabetic children was 12.9 ± 2.24 years. And the onset of diagnosis of diabetes was 5.33 ± 2.25 years old. The mean duration of diabetes was 7.59 ± 1.9 years. There were significantly higher level of HbA1c, serum Cholesterol, and serum Triglyceride, among bisphenol positive groups compared to bisphenol negative group, ($P < 0.05$). Regarding ROC curve for glucose disposal rate which shows insulin resistance it showed that the best cutoff of glucose disposal rate at 11.72 (sensitivity 75% and specificity 70.8, $P < 0.05$).

Conclusion: Significant association between BPA exposures, insulin resistance and diabetes complications in type 1 diabetic children.

Keywords: Bisphenol A, Insulin Resistance, Glycemic Control, Type 1 Diabetes Mellitus.

INTRODUCTION

Bisphenol A (BPA) is an environmental pollutant that has brought greater attention to the public health. It is one of the highest volume chemicals produced and used worldwide since 1950s and exists in human biological samples ubiquitously (Kim et al., 2013).

Bisphenol A (BPA), an endocrine disruptor (ED), has been found in more than 90% of human urine samples due to its wide use in a variety of polycarbonate plastics and epoxy resins (e.g., water bottles and plastic food containers; Lang et al., 2008).

Abundant evidence from in vivo and in vitro studies showed that BPA could affect children health (Egan, 2010), thus it has been prohibited in baby feeding bottles as they are the most susceptibility population and even a small amount of exposure would harm the babies in their later lives (Heindel et al., 2017).

Many studies have shown that BPA exposure during adulthood or

throughout life can increase type 1 D. incidence (Bodin et al., 2013; Bodin et al., 2015; Cetkovic-Cvrlje et al., 2017).

Modernization and technology, although increasing convenience, increase the exposure risk to man-made chemicals. The potential toxic effects of many of these chemicals are largely unknown. Some of these chemicals are suggested to interrupt the endocrine system and are named as “endocrine disrupting chemicals (EDCs)”. Bisphenol-A (BPA) is suggested to be an EDC and is used extensively in the manufacturing of polycarbonate plastics and epoxy resins. The main utilization of BPA is in the production of various common consumer products such as water containers, baby bottles, the resin linings of food and beverage cans, food packaging, reusable water bottles, plastic tableware, food storage containers, children’s toys and sealants in dentistry (Vandenberg, 2016 and Ahmadkhaniha et al., 2014).

Diabetes mellitus is the most commonly encountered endocrine-

metabolic disorder of childhood. It is an epidemic worldwide and might be affecting more than 300 million people. Although type 2 diabetes (T2DM) is dominating this epidemic, type 1 diabetes (T1DM) should not be overlooked because it represents 10–15% of diabetes mellitus cases (**Pociot and Lernmark, 2016**).

Among children under the age of 15 years, the risk of developing T1DM has been rapidly increasing. This rapid increase in the incidence of T1DM cannot be explained only by genetic predisposition. Environmental agents which were seldom referred to in the pathogenesis of diabetes have become suspected factors in disease development. In recent years, EDCs, including BPA, have come under suspicion as factors in diabetes development (**İnce et al., 2018**).

Studies have found some evidence that BPA exposure could contribute to the development of insulin resistance, obesity and T2DM (**Rochester, 2013, and Sun et al., 2014**).

BPA exposure can affect β -cells of the pancreas and promote autoimmunity, which accelerates insulinitis and diabetes development in an animal model of T1DM (**Bodin et al., 2013; Bodin et al., 2015**).

AIM OF THE WORK

The aim of our study was to assess the association between BPA exposures by measuring urinary levels of BPA, insulin resistance using estimated glucose disposal rate, glycemic control using mean value of HbA1C.

Ethical Considerations:

1. Approval of ethical committee in the university was obtained before the study.
2. Full informed written consent was taken from parents.
3. Any risks during the course of the research were cleared to the participants and to the Ethical Committee on time.
4. Privacy of participants and confidentiality of the data were maintained.
5. The patient has the right to withdraw from the study at any time.
6. The authors declared that there is no conflict of interest or any financial support regarding the study or publication.

PATIENTS AND MATERIALS

This study is a descriptive cross-sectional study that was carried out on children with type 1 DM attending to Pediatric Endocrinology Outpatient Clinic,

Abo Elrish Pediatric Hospital, Cairo University, and Pediatric Endocrinology Outpatient Clinic of EL-Hussein University Hospital, Al Azhar University Egypt.

Inclusion criteria were:

1. Children with type 1 DM (according to ADA criteria) of at least 4 years duration.
2. Children age 6-18 years old (males and females).

Exclusion criteria were:

1. Newly diagnosed diabetics.
2. Other types of diabetes. Patients with T2DM, MODY, and syndromic diabetes (Down, Wolfram syndrome, etc.).
3. Children have other chronic significant health problems (including other autoimmune diseases).

All the studied children were subjected to:

I: Detailed History taking, Stressing On:

- Age and sex of the patient, Age of onset of Diabetes and Diabetes therapy.
- History of bisphenol exposure e.g: ingestion of food and drinks which has been stored or reheated in plastics, cans, and food packaging materials.

- Family history of diabetes (type 1 or type 2).
- Symptoms of peripheral neuropathy: pain, burning, tingling and numbness and loss of protective sensation.

II: Complete physical examination. Stressing On:

- Vital signs: Heart rate, respiratory rate, temperature, blood pressure.
- Anthropometric measurements: Height, weight, Body mass index, Waist circumference, Head circumference, and hip circumference.
- Neurological examination: to detect any evidence of peripheral neuropathy:

III: Investigations: Laboratory investigations including:

- HbA1C (%): (reference values between 4.7 and 6.0)
- Lipid profile (LDL, TG, and Cholesterol).
- Albumin/ creatinin ratio (A/C ratio)
- Early morning urine sample was taken for Urinary BPA levels of all children were measured using high-performance liquid chromatography. To measure urinary BPA concentration,

morning spot urine sample will be collected from each study participant. A comprehensive quality control system, including reagent blanks, will be used to ensure that samples will not be contaminated during handling, storage, and analysis.

VI: Estimation of glucose disposal rate using formula:

$eGDR \text{ (mg/kg/min)} = 24.31 - (12.22 * \text{Waist/Hip Ratio}) - (3.29 * \text{HTN}) - (0.57 * \text{HbA1c})$.

According to the results our studied cases were classified into two groups:

- Bisphenol positive group (52 patients)
- Bisphenol negative group (48 patients).

Statistical Analysis:

Statistical Package for Social Science (SPSS v20) was used after transforming the data from Excel 2013 sheet. Categorical variables were presented by number and percent. They were compared using Chi-square test or Fischer's

exact test when appropriate. Continuous variables were presented by mean and standard deviation or median and range. They were compared by student's t-test if parametric data and using Mann Whitney U test if non parametric data. In all tests, P value was considered significant if less than 0.05. 4- The ROC (receiver operating characteristic) curves, this procedure used to evaluate the performance of classification schemes in which there is one variable of two categories by which subjects are classified. They were constructed by calculating the sensitivities and specificities of the variable. The cutoff value with the highest accuracy was selected as the diagnostic cutoff points.

The point of the curve closest to the upper left corner presents the point at which the ability of the test is maximized and the number of erroneous diagnoses is minimized.

RESULTS

Our results will be demonstrated in the following table and figures:

Table (1): Sociodemographic and clinical characteristics of the studied children

Characteristics	
Age (years)	
Mean±SD	12.9±2.24
Age at diagnosis (years)	
Mean±SD	5.33±2.25
Diabetes duration (years)	
Mean±SD	7.59±1.9
Gender	N (%)
Male	51 (51%)
Female	49 (49%)
Consanguinity	N (%)
Positive	26 (26%)
Negative	74 (74%)
Family history of diabetes	N (%)
Positive	8 (8%)
Negative	92 (92%)
Weight (kg)	
Mean±SD	43.2±11.4
Height (cm)	
Mean±SD	148.6±10.3
(BMI) (kg/m²)	
Mean±SD	19.3±3.7
Waist hip ratio	
Mean±SD	0.74±0.043

Regarding anthropometric parameters (Wt, Ht, BMI and waist hip ratio) the mean value

are within normal range according to mean age.

Table (2): Correlation between Sociodemographic characteristics and bisphenol result

Bisphenol	Positive N=52		Negative N=48		P-Value
	Mean	±SD	Mean	±SD	
Parameters					
Age (Years)	12.7	2.15	13.05	2.32	0.52 * NS
Height (cm)	147.6	9.79	149.4	10.76	0.39 *NS
Weight (kg)	41.3	7.67	44.9	13.8	0.11 *NS
BMI	18.7	1.94	19.8	4.79	0.17 * NS
waist/hip ratio	0.74	0.027	0.74	0.054	0.63 * NS
Gender	N	(%)	N	(%)	
Male	26	50.0	25	52.1	0.48 * NS
Female	26	50.0	23	47.9	

This table shows no statistically significant difference regarding sociodemographic in

bisphenol positive and negative group, ($P>0.05$).

Table (3): Correlation between diabetes complications, laboratory results and urinary bisphenol

Bisphenol	Positive N=52		Negative N=48		P-Value
	Mean	±SD	Mean	±SD	
Parameters					
HBA1c (%)	7.013	0.79	5.8	0.56	<0.001 S
Glucose disposal rate (mg/kg/min)	8.25	0.65	11.108	1.1	<0.001 S
Cholesterol(mg/dl)	209.673	52.7	152.1	37.35	<0.001 S
Triglyceride(mg/dl)	164.327	59.4	116.4	39.45	<0.001 S
Diabetes complications					
	N	%	N	%	
Micro albuminuria	10	19.23	2	4.1	0.003 S
Hypertension	3	5.8	0	0.0	<0.001 S
Peripheral neuropathy	2	3.8	0	0.0	<0.001 S
Retinopathy	2	3.8	0	0.0	<0.001 S

Regarding the long term diabetes complications there was higher significant difference

between positive bisphenol group and negative group, ($P<0.05$).

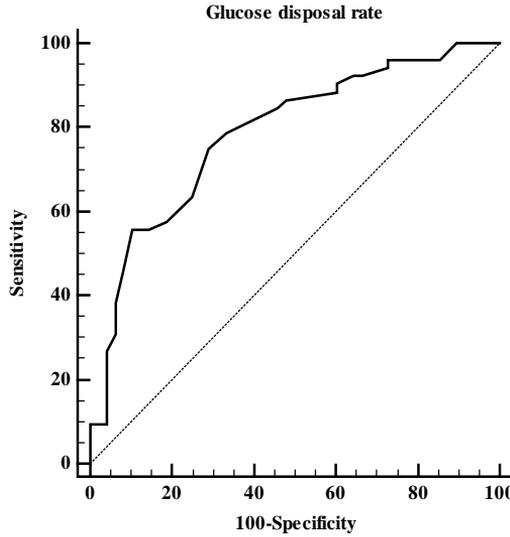


Figure (1): ROC (receiver operating characteristic) curve for glucose disposal rate

ROC curve for glucose disposal rate which show insulin resistance with the best cutoff rate at 11.72 with sensitivity 75% and specificity 70.8, $P < 0.05$.

Table (4): Sensitivity and specificity of glucose disposal rate

Marker	Area under the Curve (95% CI)	Significance	Cutoff	Sensitivity	Specificity
Glucose disposal rate	0.776 (0.692 to 0.861)	<0.05 S	11.72	75%	70.8%

ROC: Receiver operating characteristic curves,

DISCUSSION

In our study, the frequency of normal weight diabetic children was 60% and only 5% were obese at the study time. This finding was similar to **ISPAD (2000)** and **Ismail et al. (2008)** where they stated that impaired growth and

delayed puberty may not be associated with diabetes mellitus.

In our study there was a significant and positive correlation were found between BPA concentrations and prevalence of T1DM. Our finding is in agreement with **Shankar et al., 2011** and **Silver et al. 2011** who

have showed strong positive associations between the urinary concentrations of BPA and diabetes in adults. Whereas in disagreement with **Kilpatrick et al., (2009)** which reported that there is no association between urine BPA levels and diabetes.

In another study, Lang et al. used NHANES (National Health and Nutrition Examination Survey) data from 2003/2004 and found that higher BPA concentrations in urine were associated with diabetes and cardiovascular diagnoses, but not with other common diseases (**Lang et al., 2008**).

Melzer, (2008) then analyzed NHANES data from a subsequent survey, from 2005/06, and found that in those years, BPA levels were lower than they had been in 2003/2004. The association between BPA and diabetes was significant in pooled data (2003–2006), but did not reach significance in 2005/06 alone.

In our study regarding correlation between bisphenol and serum Cholesterol and serum Triglyceride there were higher significant difference among bisphenol positive groups compared to bisphenol negative group, $P < 0.05$, In agreement with **Diana et al., 2019** findings.

However, a cross-sectional study conducted in Spain ($n = 115$, median age 12.6 years (10.5–15.4), did not find a correlation between bisphenol positive values and lipid levels (**Atance et al., 2013**).

In our study, the optimal cut-off value of glucose disposal rate whether presence of bisphenol or not with the best cutoff of glucose disposal rate at 11.72 (sensitivity 75% and specificity 70.8, $P < 0.05$). For comparison, in another study (**Chillarón et al., 2009**), an eGDR value < 8.77 had 100% sensitivity and 85.2% specificity for the diagnosis of bisphenol intoxication in T1DM patients. This difference may be due to poor glycemic control in our patients.

Also, the T1DM group with bisphenol negative in urine was found to have a higher mean GDR (11.108 ± 1.1034) when compared to bisphenol positive group (8.25 ± 0.65) indicating higher insulin resistance in patients with bisphenol positive. indicating negative correlation between GDR and insulin resistance. Similar to **Chillarón et al., (2009)** where GDR was higher in patients with bisphenol negative compared with those bisphenol positive, indicating higher IR in patients with chronic complications.

In our study microalbuminuria was the most common microvascular complication in the studied group of adolescents with T1DM and it was present in 12% (12 patients). This is consistent with the study done by **Chillarón and co-workers (2013)** who stated that Diabetic nephropathy is the most frequently observed microvascular complication in adolescents with T1DM and the one with the earliest onset.

In earlier studies, microalbuminuria rates were reported to vary between 3% and 25.4%, depending on diabetes duration (**Harjutsalo et al., 2011**).

While, **Ismail et al., (2008)** reported a prevalence of microalbuminuria was 9.6% in their study.

Gupta et al. reported that Microalbuminuria was present in 38% and Normo-albuminuria in 62% of the studied diabetic patients (**Gupta et al., 2013**).

In our study prevalence of peripheral neuropathy was found to be 2% (2 patients) among studied children. This is in agreement with **Ismail et al., (2008)** who reported a prevalence of neuropathy 3.1% in their study.

In our study retinopathy prevalence was 2% (2 patients). This result is comparable to

Ismail et al. (2008) who reported incidence of retinopathy 4.1% in their study.

CONCLUSION

Our results come to prove the toxic effects of BPA on the children with type 1 DM, on diabetes complications (microalbuminuria, diabetes retinopathy, diabetes neuropathy and hypertension), insulin resistance and poor glycemic control.

RECOMMENDATION

1. Increase the public awareness about the adverse harmful health effect of BPA including its effect on the health of children its common sources of exposure to avoid its toxic effect.
2. All plastic feeding packing materials should be free from epoxy resin and other harmful types of plastics to avoid harmful effect.
3. Health strategies should be implemented to reduce BPA consumption from different sources to mitigate its adverse effects on children with type 1 DM.

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الترابط بين مستويات البيسفينول في البول ، ومقاومة الأنسولين، والتحكم في نسبة السكر في الدم ومضاعفات مرض السكري لدى الأطفال المصابين بالنوع الأول من داء السكري

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البيسفينول موجود في كل مكان في بيئتنا، كما يتضح من حقيقة أن أكثر
من 90% من الأفراد لديهم مستويات قابلة للاكتشاف من BPA موجودة في
بولهم، وهو الطريق الأساسي للإفراز عند البشر. تم العثور على البيسفينول في
حديثي الولادة والأطفال والبالغين، ويمكن قياسه في مجموعة من سوائل
وأنسجة الجسم، بما في ذلك البول والدم واللعاب وأنسجة المشيمة والأنسجة
الدهنية وحليب الثدي.

الأهداف: الهدف من هذه الدراسة هو تقييم العلاقة بين التعرض للبيسفينول عن
طريق قياس المستويات البولية لمادة البيسفينول ومقاومة الأنسولين باستخدام
معدل التخلص المقدر من الجلوكوز، والتحكم في نسبة السكر في الدم باستخدام
القيمة المتوسطة للهيموجلوبين السكري ونسبة الجلوكوز بالدم أثناء الصيام
وبعد الأكل.

الموضوعات والطرق: دراسة مقطعية وصفية أجريت على الأطفال المصابين
بالنوع الأول من السكري المترددين على عيادة الغدد الصماء والسكر عند

الأطفال، في مستشفى الأطفال بأبو الريش، جامعة القاهرة، مصر وكذا عيادة الغدد الصماء والسكر عند الأطفال، في مستشفى الحسين الجامعي، جامعة الأزهر، مصر. اشتملت الدراسة على أطفال مصابين بالأنواع الأولى من السكري تتراوح أعمارهم بين 6 و 18 عامًا.

النتائج: كان متوسط عمر الأطفال المشاركين في البحث يتراوح ± 12.9 سنة. وكان متوسط العمر عند التشخيص بالسكر 2.25 ± 5.33 سنة. وكانت متوسط مدة مرض السكري لديهم 1.9 ± 7.59 سنة. كانت البيانات المخبرية مثل الهيموجلوبين السكري، ومعدل التخلص من الجلوكوز، وكوليسترول الدم، والدهون الثلاثية أعلى بين المجموعات الإيجابية للبيسفينول مقارنة بالمجموعة السلبية للبيسفينول، وكانت ذو دلالة احصائية.

منحنى ROC للتمييز بين معدل التخلص من الجلوكوز سواء كان وجود بيسفينول أم لا مع أفضل معدل قطع للتخلص من الجلوكوز عند 11.72 (حساسية 75% ونوعية 70.8، وكانت ذو دلالة احصائية $P < 0.05$).

استنتاجات البحث:

صُنّف الأطفال المصابون بداء السكري إلى مجموعتين من حيث وجود أو عدم وجود البيسفينول في عينات البول إلى 52 طفلاً إيجابياً للبيسفينول و 48 طفلاً سلبياً من مادة البيسفينول.

كانت البيانات المخبرية مثل الهيموجلوبين السكري، ومعدل التخلص من الجلوكوز، والكوليسترول في الدم، و الدهون الثلاثية أعلى بين المجموعات الموجبة للبيسفينول مقارنة بالمجموعة السالبة للبيسفينول وكان الفرق ذو دلالة احصائية.

تم استخدام معدل التخلص من الجلوكوز لتبيين وجود بيسفينول في البول أم لا بواسطة اختبار معدل منحنى قطع التخلص من الجلوكوز وكانت افضل معدل هو عند 11.72، وكانت حساسية الاختبار عند 75% ونوعيته عند 70.8، وكانت نتيجة هذا الاختبار لها دلالة احصائية.

توصيات البحث:

1. زيادة الوعي العام بالآثار الصحية الضارة للبيسفينول بما في ذلك تأثيرها على صحة الأطفال من المصادر الشائعة لتعرضها لتجنب تأثيرها السام.
2. يجب أن تكون جميع مواد التعبئة والتغليف البلاستيكية خالية من راتنجات الايبوكسي وأنواع البلاستيك الضارة الأخرى لتجنب تأثيرها الضار.
3. يجب تنفيذ استراتيجيات صحية لتقليل استهلاك البيسفينول من مصادر مختلفة للتخفيف من آثاره السلبية على الأطفال المصابين بالنوع الأول من السكر.