Urinary Excretion of 11 dehydrothromboxane B_2 in Diabetic Children Relation to Clinical and Biochemical Parameters

Prof.Medhat H. Shehata[1], Prof. Mona H. El-Samahy[2], Dr.Hanan H. Shehata[3], Dr.Inas S. Nabeeh[3], Dr.Nevine G. Andrawes[4], Dr.Iman H. Kamel[5], and Usama Farouk Fikry
[1]Professor of Pediatrics Institute of Postgraduate Childhood Studies Ain Shams University, [2]Professor of Pediatrics Faculty Of Medicine Ain Shams University, [3]Assistant Prof. of Med.Biochemistry& Mol.Biology Faculty of Medicine Ain Shams University, [4]Assistant Prof. of Pediatrics, Faculty of Medicine Ain Shams University, [5]Assistant Prof. of child health and nutrition, National Research Center

Abstract

Diabetes is the 6th most important cause of disability burden in Egypt. Cardiovascular diseases are the leading cause of death in diabetics. Oxidative stress is involved in β- cell destruction and is recognized as a mediator in the development of macrovascular or cardiovascular complications in type 1 diabetes mellitus. Products of arachidonic acid metabolism elicit inflammatory responses and diseases in diabetic children such as atherosclerosis. Hyperglycemia induced activation of thromboxane pathway evidenced by increase urinary excretion of 11-dehydrothromboxane B2 (indicator of oxidative stress). This study measured urinary excretion of 11 dehydro- thromboxane B2 in 40 type 1 diabetic children (12.38± 2.75) years and 40, age and gender matched, healthy controls (10.88± 3.23) years. Mean urinary 11 dehydrothromboxane B2 concentrations showed statistical significant difference between diabetic group (1884.8± 826.86 pg/mg creatinine) and controls (601.95± 229.24 pg/mg creatinine, p<0.001). Also, total cholesterol (183.75± 30.47 versus 112.6± 27.07 mg/dl, p<0.001), triglycerides (147.45± 29.91 versus 73.08± 13.3, p<0.001), HDL (34.8± 4.95 versus 45.6± 8.25 mg/dl, p<0.001), LDL (120.9± 30.5 versus 83.6± 24.2 mg/dl, p<0.001), HbA1C (11.48± 1.79 versus 5.37± 0.59, p<0.001) and fasting C- peptide (0.33± 0.14 versus 2.05± 0.87, p<0.001) showed statistical significant difference between diabetic children and adolescents and healthy controls. Our results showed also significant positive correlation between urinary 11- dehydrothromboxane B2 and HbA1c (r= 0.627, p= 0.012), triglycerides (r= 0.520, p= 0.047) and total cholesterol (r= 0.668, p= 0.007). In conclusion, the increase of triglycerides and LDL- cholesterol levels in our study confirmed the dyslipidemia pattern in pediatric type 1 DM patients. Our results confirmed that hyperglycemia induced activation of thromboxane pathway in type 1 diabetic children and adolescents as evidenced by increase urinary excretion of the indicator of oxidative stress status 11- dehydrothromboxane B2. Also, we showed a significant positive correlation between the urinary excretion of 11- dehydrothromboxane B2 and the laboratory parameters of lipid metabolism. Therefore, urinary 11- dehydrothromboxane B2 can be used as a potential non invasive biomarker of dyslipidemia in type 1 diabetic children.

علاقة الإفراز البولى لمادة ١١ ديهيدروثرومبوكسان ب٢ بتمثيل الدهون عند الأطفال المصابين بالسكر من النوع الأول

ان مرض السكر من النوع الأول هو أكثر أمراض الغدد الصماء والأيض شيوعاً، أثناء فترة الطفولة والمراهقة، وله آثار هامة على النمو البدني. مضاعفات مرض السكر تمثل عبئا كبيرا على الخدمات الصحية والمريض. وجد ان أمراض القلب والأوعية الدموية هى السبب الرئيسى للوفاة فى مرضى السكرى. وتشارك الاكسدة فى تدمير خلايا البنكرياس كما انها تسبب مضاعفات الأوعية الدموية والقلب فى مرضى السكرى من النوع الاول.

تهدف هذه الدراسة إلى تحديد مستوى ١١ ديهيدروثرومبوكسان ب٢ فى البول لدى الأطفال والمراهقين الذين يعانون من مرض السكر من النوع الأول كدلالة للاكسدة.

أجريت هذه الدراسة على ٤٠ من الأطفال والمراهقين الذين يعانون من مرض السكر من النوع الأول وأشتملت ايضا الدراسة على مجموعة ضابطة مكونة من ٤٠ من الأطفال والمراهقين الأصحاء متطابقى العمر والنوع مع المجموعة المريضة. وقد خضع المشاركين فى البحث لأختبارات معملية تشمل قياس مستوى الهيموجلوبين السكرى فى الدم، الدهون الثلاثية والكوليسترول الكلى وكل من الكوليسترول مرتفع ومنخفض الكثافة وبالإضافه الى قياس نسبة افراز ١١ ديهيدروثرومبوكسان ب٢ فى البول.

وبمقارنة المجموعتين، وجد فارق ملحوظ بينهما فى مستوى ١١ ديهيدروثرومبوكسان ب٢ فى البول كما وجدت الدراسة فارق ملحوظ فى مستوى الدهون الثلاثية والكوليسترول الكلى وكل من الكوليسترول مرتفع ومنخفض الكثافة بالدم، وجدت الدراسة علاقة ايجابية وثيقة بين مستوى ١١ ديهيدروثرومبوكسان ب٢ فى البول وبين نسب الهيموجلوبين السكرى، الدهون الثلاثية والكولسترول الكلى.

تدل نتائج هذا البحث على أن ارتفاع نسبة السكر لدى الأطفال والمراهقين الذين يعانون من مرض السكر من النوع الأول يؤدى الى تحفيز تصنيع الثرومبوكسان بدلالة زيادة الافراز البولى لمادة ١١ ديهيدروثرومبوكسان ب٢، كما اثبتت النتائج وجود علاقة ايجابية وثيقة بين مستوى ١١ ديهيدروثرومبوكسان ب٢ والاختبارات المعملية لتمثيل الدهون، من ثم يمكن قياس مستوى ١١ ديهيدروثرومبوكسان ب٢ فى البول دلالة لسوء تمثيل الدهون لدى الأطفال والمراهقين الذين يعانون من مرض السكر من النوع الأول.

Introduction:

Diabetes is the 6th most important cause of disability burden in Egypt. It is estimated that by the year 2030, Egypt will have 8.6 million persons with diabetes (Shaw et al., 2010). Cardiovascular diseases are the leading cause of death in diabetics (Maahs 2008). Oxidative stress is involved in β- cell destruction (Kaneto et al., 2007) and is recognized as a mediator in the development of macrovascular or cardiovascular complications in type 1 DM (Wegner et al, 2011).

Products of arachidonic acid metabolism elicit inflammatory responses and diseases such as atherosclerosis (Rama and Jerry, 2004). Urinary excretion of 11- dehydrothromboxane B2 (11- dTXB2), is an index of endogenous thromboxane A2 production (Aldo et al, 1995). It can be used to evaluate oxidative stress (Boizel et al, 2010 and Viviana, 2010).

Our aim was to evaluate oxidative stress in Egyptian diabetic children and adolescents, through the measurement of urinary excretion of 11 dehydrothromboxane B2 in 40 type 1 diabetics and 40, age and gender matched, healthy controls. Furthermore, its relation to parameters of lipid metabolism was evaluated in type 1 diabetics.

Subjects And Methods:

This study was conducted on 40 children and adolescents with uncomplicated T1DM recruited from Diabetes Clinic, Children's Hospital, Ain Shams University. They were 19 males (47.5%) and 21 females (52.5%). Their age ranged from (6.5- 16) years with a mean age of 12.38± 2.75 years. Duration of diabetes ranged from (5- 13) years (8.9± 2.3) years. The control group consisted of 40 healthy children and adolescents matched in age and gender to the study group. They were 16 males (40.0%) and 24 females (60.0%). Their age ranged from (6- 16) years with a mean age of (10.88± 3.23) years. All participants were subjected to history taking and thorough clinical examination. The entire protocol was approved by institutional ethical committee. All parents or care givers provided signed informed consent for participation in the study as required.

Samples Collection And Processing:

Venous blood samples (5 ml) were aseptically withdrawn from both groups after an overnight fasting for (12- 14) hours and divided into two portions as follows: 2.0 ml of blood was placed in an EDTA containing tube for the determination of glycated hemoglobin (HbA1c) using Helena GLYCO- Tek affinity column method (Helena Laboratories, Beaumont, Texas, USA). The remaining 3.0 ml of blood was used for separation of serum. The separated serum samples were kept frozen at -80ºC until used in the determination of total cholesterol, HDL- cholesterol and triglycerides according to the manufacturers' instructions of standard enzymatic kits (Randox Laboratories, Crumlin, UK), C-peptide according to the manufacturer's instructions of IBL ELISA kit (Immuno- Biological Laboratories Inc., Minnesota, USA). LDL-cholesterol levels calculated with the Friedewald equation. Urinary excretion of 11 dehydrothromboxane B2 was determined according to the manufacturer's instructions of 11- dehydrothromboxane B2 EIA kit, Cayman chemicals, USA. Urinary 11- dehydrothromboxane B2 concentrations were normalized for urinary creatinine concentration and the results were expressed in pg 11 dhTXB2/ mg creatinine.

Statistical Analysis:

The analysis was done using the Statistical Package for the Social Sciences (SPSS software version 19, SPSS Inc., Chicago, IL). Results were expressed as means± standard deviation (SD). Differences between continous variables were analyzed using Student's t- test. Correlation between different variables was performed by Pearson. Statistical significance was set at a value of $p<0.05$.

Results:

The vital signs and anthropometric measures of all participants are shown in Table (1). There was statistically high significant difference in the mean Levels of urinary 11- dehydrothromboxane B2 ($p<0.001$), total cholesterol ($p<0.001$), triglycerides ($p<0.001$), HDL ($p<0.001$), LDL ($p<0.001$), HbA1C ($p<0.001$) and C-peptide ($p<0.001$) between healthy controls and diabetic children and adolescents Table (2). The correlation coefficients between urinary 11- dehydrothromboxane B2 and the investigated laboratory parameters revealed significant positive correlation between urinary 11-dehydrothromboxane B2 and HbA1c ($r= 0.627$, $p= 0.012$), triglycerides ($r= 0.520$, $p= 0.047$) and total cholesterol ($r= 0.668$, $p= 0.007$), Table (3).

Table (1) Vital signs and anthropometric measures of the studied groups

Parameters	Diabetic Group Mean± SD (range)	Control Group Mean± SD (range)	t	p
Pulse	87.50± 8.00 (70- 100)	86.25± 8.89 (75- 105)	- 0.66	0.511
Systolic BP	107.25± 8.77 (100- 130)	109.25± 7.56 (100- 120)	1.093	0.278
Diastolic BP	71.00± 7.36 (60- 85)	71.63± 5.24 (60- 80)	0.438	0.663
Weight (Kg)	47.59± 17.05 (22- 76)	33.58± 6.72 (22- 49)	- 4.96	<0.001**
Height (M)	1.46± 0.16 (1.17- 1.67)	1.40± 0.13 (1.14- 1.60)	- 2.077	0.041*
BMI (Kg/m2)	21.77± 4.01 (14.7- 28.6)	17.13± 1.91 (13.4- 22.37)	- 6.614	<0.001**
Waist Circumference (Cm)	67.18± 8.83 (52- 80)	61.13± 4.59 (54- 70)	- 3.844	<0.001**

*p<0.05 is significant, **p<0.01 is highly significant.

Table (2) Laboratory results of the studied groups

Parameters	Diabetic Group Mean± SD (range)	Control Group Mean± SD (range)	t	p
Triglycerides (mg/dl)	147.45± 29.91 (98- 205)	73.08± 13.3 (57- 98)	- 14.37	<0.001**
Total cholesterol (mg/dl)	183.75± 30.47 (145- 266)	112.6± 27.07 (61- 145)	- 10.932	<0.001**
HDL (mg/dl)	34.8± 4.95 (24- 40)	45.6± 8.25 (34- 63)	7.589	<0.001**
LDL (mg/dl)	120.9± 30.5 (66- 160)	83.6± 24.2 (50- 136)	- 7.273	<0.001**
Hba1c (%)	11.48± 1.79 (9.0- 16.0)	5.37± 0.59 (4.4- 6.2)	- 20.642	<0.001**
Fasting C-peptide (ng/ml)	0.33± 0.14 (0.1- 0.7)	2.05± 0.87 (0.5- 3.2)	5.063	<0.001**
Urinary 11- Dtxb2 (pg/mg creatinine)	1884.8± 826.86 (660- 3935)	601.95± 229.24 (277- 1170)	- 9.456	<0.001**

**p<0.01 is highly significant.

Table (3) Correlation between Urinary 11- dTXB2 and the studied parameters

Parameters	Diabetic Group		Control Group	
	r	p	r	p
Age (Years)	-0.107	0.505	0.201	0.213
Weight (Kg)	-0.008	0.958	0.119	0.465
Height (M)	-0.07	0.662	0.216	0.181
BMI (Kg/m2)	-0.076	0.636	0.059	0.717
Pulse	-0.099	0.542	0.129	00.428
Systolic Blood Pressure	-0.152	0.349	-0.404	0.135
Diastolic Blood Pressure	0.098	0.546	-0.154	0.343
Hba1c (%)	0.627*	0.012	-0.206	0.202

Parameters	Diabetic Group		Control Group	
	r	p	r	p
TG Level	.520*	0.047	0.139	0.394
Total Cholesterol	0.668**	0.007	-0.0167	0.302
HDL	0.204	0.208	- 0.115	0.480
LDL	0.080	0.625	0.421**	0.007

* Correlation is significant at the 0.05 level. **Correlation is highly significant at the 0.01 level.

Discussion:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Ramakrishna and Jailkhani, 2007).

Thromboxane A2 is a member of eicosanoid family of compounds derived in mammals from arachidonic acid. Emerging evidence demonstrated that estimation of human urinary 11- dehydrothromboxane B2 can be used to evaluate oxidative stress (Viviana,2010). Therefore, we evaluated urinary excretion of 11- dehydrothromboxane B2 in type 1 diabetic children as an index of dyslipidemia through its assosiation to laboratory parameters of lipid metabolism.

Regarding laboratory data, mean serum levels of cholesterol and triglycerides, in this study, were 183.75± 30.47 mg/dl and 147.45± 29.91 mg/dl respectively with statistical high significant difference between diabetic and healthy groups (p= 0.000); in agreement with Erciyas et al, 2004 and Petitti et al., 2007 who found high total cholesterol levels in diabetic children compared to healthy controls. Also, Uttra et al., 2011 has reported that 58% patients of type 1 DM were found to be hyperlipidemic.

Significantly lower mean HDL levels was found in diabetic patients (34.8± 4.95 mg/dl) compared to that of controls (45.6± 8.25 mg/dl, p= 0.000). Meanwhile, higher mean LDL levels was found in diabetic patients (120.9± 30.5 mg/dl) compared to that of controls (83.6± 24.2 mg/dl, p= 0.000). This came in concordance with Levy, 2011. The increase of triglycerides and LDL-cholesterol levels confirmed the dyslipidemia pattern in pediatric type 1 DM patients. LDL data agree with Ercyias et al. (2004) who stated that LDL oxidation might decrease catabolism of LDL- cholesterol, thus causing increase in LDL- cholesterol levels and attributed LDL increase to decreased activity of cholesterol ester transfer protein and lipoprotein lipase activity.

Hyperglycemia contributes to platelets reactivity through direct effects and by promoting glycation of platelets proteins. Persistent platelet activation was reflected by enhanced 11- dehydrothromboxane B2 excretion, in both type 1 and 2 diabetes mellitus (Davi et al, 1990, Davi et al, 1999). In the present study, estimation of mean urinary 11 dehydrothromboxane B2 showed statistical significant difference between type 1 diabetic patients (1884.8± 826.86 pg/mg creatinine) and healthy controls (601.95± 229.24 pg/mg creatinine, p= 0.000); in agreement with Davì et al., 1990, Davì et al, 1999, Davì et al, 2003 and Boizel et al, 2010 who confirmed persistent platelet activation in diabetics and enhanced peroxidation of arachidonic acid to form isoprostanes, including 11- dehydrothromboxane B2.

In our study, we found that HbA1c was higher in diabetic group compared to healthy group (11.48± 1.79% versus 5.37± 0.59%, p= 0.000). A positive significant correlation was found between urinary 11 dehydrothromboxane B2 and HbA1c (r= 0.627, p= 0.012). Similar to the relation between HbA1c and biomarkers of oxidative stress, Varashree and Bhat (2011) stated that the biomarker of lipid peroxidation (MDA) was positively correlated with HbA1c.

Although no enough studies were found as regards urinary 11 dehydro-thromboxane B2 concentrations in type 1 DM, Gonçalves et al (2014) found that the use of Metformin, in type 2 diabetics who were taking daily 100 mg acetyl salicylic acid (ASA) for 15 days, caused a reduction of urinary 11-dehydrothromboxane B2 above 75% through improvement of oxidative stress and control of platelet activation, potentially reducing cardiovascular risk. Meanwhile, Ames et al, 2012 suggested that oxidative stress may maintain platelet function irrespective of cyclooxygenase- 1 (COX- 1) pathway inhibition and/or increase systemic generation of thromboxane from non-platelet sources.

In our study, a positive correlation was found between urinary 11-dehydrothromboxane B2 and triglycerides (r= 0.520, p= 0.047) and cholesterol (r= 0.668, p= 0.007) in diabetic children and adolescents. No sufficient data was found regarding these correlations.

In conclusion, hyperglycemia induced activation of thromboxane pathway in type 1 diabetic children and adolescents evidenced by increase urinary excretion of the indicator of oxidative stress status 11- dehydrothromboxane B2 and was significantly correlated to laboratory parameters of lipid metabolism. Therefore, urinary 11- dehydrothromboxane B2 can be used as a potential non invasive biomarker of dyslipidemia in type 1 diabetic children.

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