**Urinary Excretion of 11 dehydrothromboxane B2 in Diabetic Children: Relation to Clinical and Biochemical Parameters**

A Thesis proposal Submitted for fulfillment of ph. D degree in Childhood Studies (Child Health and Nutrition) Department of Medical Studies for Children

By

**Usama Farouk Fikry**

MBBCh, Msc in pediatrics

**Supervised by**

**Dr. Medhat Hassan Shehata**  **Dr. Mona Hussein El-Samahy** Professor of Pediatrics Professor of Pediatrics Institute of Postgraduate Head of Pediatric Diabetes Unit

Childhood Studies Faculty of Medicine

Ain Shams University Ain Shams University

**Dr. Hanan Hussein Shehata Dr. Nevine Gamal Andrawes** Assistant Prof.of Medical Biochemistry Assistant Prof. of Pediatrics

and Molecular Biology Faculty of Medicine Faculty of Medicine Ain Shams University

Ain Shams University

 **Dr. Iman Hussein Kamel**

Assistant Prof.of child health and nutrition

National Research Center

**Institute of postgraduate childhood studies**

**Ain Shams University**

**2015**

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

**مدحت حسن شحاتة١، منــى حسين السماحى٢، نيفين جمال اندراوس٢، حنان حسين شحاتة٣، ايمان حسين كامل٤، ٳيناس سمير نبيه٣، أسامة فاروق فكرى١**

**١قسم الدراسات الطبية معهد الدراسات العليا للطفولة جامعة عين شمس**

**٢قسم الاطفال كلية الطب جامعة عين شمس**

**٣قسم الكيمياء الحيوية كلية الطب جامعة عين شمس**

**٤قسم صحة وتغذية الطفل المعهد القومى للبحوث**

**الملخص العربى**

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 هي السبب الرئيسي للوفاة في مرضى السكرى. وتشارك الاكسدة في تدمير خلايا البنكرياس كما انها تسبب مضاعفات الأوعية الدموية والقلب في 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 و أشتملت ايضا الدراسة على مجموعة ضابطة مكونة من ٤٠ من الأطفال والمراهقين الأصحاء متطابقى العمر و النوع مع المجموعة المريضة. و قد خضع المشاركين فى البحث لأختبارات معملية تشمل قياس ​​مستوى الهيموجلوبين السكرى في الدم، الدهون الثلاثية و الكوليسترول الكلى و كل من الكوليسترول مرتفع و منخفض الكثافة و بالإضافه الى قياس نسبة افراز ١١ ديهيدروثرومبوكسان ب٢ فى البول.

و بمقارنة المجموعتين ، وجد فارق ملحوظ بينهما فى مستوى ١١ ديهيدروثرومبوكسان ب٢ فى البول كما وجدت الدراسة فارق ملحوظ فى مستوى الدهون الثلاثية و الكوليسترول الكلى و كل من الكوليسترول مرتفع و منخفض الكثافة بالدم٠ وجدت الدراسة علاقة ايجابية وثيقة بين مستوى ١١ ديهيدروثرومبوكسان ب٢ فى البول و بين نسب الهيموجلوبين السكرى، الدهون الثلاثية و الكولسترول 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 ب٠٢ كما اثبتت النتائج وجود علاقة ايجابية وثيقة بين مستوى ١١ ديهيدروثرومبوكسان ب٢ و الاختبارات المعملية لتمثيل الدهون٠ 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.

# 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.

**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) | -.107 | 0.505 | 0.201 | 0.213 |
| Weight (Kg) | -.008 | 0.958 | .119 | .465 |
| Height (M) | -.07 | 0.662 | .216 | .181 |
| BMI (Kg/m2) | -.076 | 0.636 | .059 | .717 |
| Pulse | -.099 | 0.542 | .129 | 0.428 |
| Systolic blood pressure | -.152 | 0.349 | -.404 | .135 |
| Diastolic blood pressure | .098 | 0.546 | -.154 | 0.343 |
| HbA1c (%) | **.627\*** | **0.012** | -.206 | 0.202 |
| TG level | **.520\*** | **0.047** | .139 | 0.394 |
| Total cholesterol | **.668\*\*** | **0.007** | -.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.

**REFERENCES**

**1) Aldo F, Vincent PF. (1995):** Mass spectrometric evidence for the anomalous chemical behavior of 11-dehydrothromboxane B2. Chemistry and physics of lipids 77: 33-40.

**2) Ames PR, Batuca JR, Muncy IJ, De La Torre IG, Pascoe-Gonzales S, Guyer K, Matsuura E, Lopez LR. (2012):** Aspirin insensitive thromboxane generation is associated with oxidative stress in type 2 diabetes mellitus. Thromb Res. 130(3):350-354.

**3) Boizel R, Bruttmann G, Benhamou PY, Halimi S, Stanke-Labesque F. (2010):** Regulation of oxidative stress and inflammation by glycaemic control: evidence for reversible activation of the 5-lipoxygenase pathway in type 1, but not in type 2 diabetes. Diabetologia 53(9):2068-2070.

**4) Davì G, Catalano I, Averna M, et al. (1990):** Thromboxane biosynthesis and platelet function in type II diabetes mellitus. N Engl J Med. 322: 1769-1774.

**5) Davì G, Ciabattoni G, Consoli A, et al. (1999):** In vivo formation of 8-iso-PGF2 and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. Circulation. 99:224–229.

**6) Davì G, Chiarelli F, Santilli F, Pomilio M, Vigneri S (2003):** Enhanced Lipid Peroxidation and Platelet Activation in the Early Phase of Type 1Diabetes Mellitus: Role of Interleukin-6 and Disease Duration.Circulation*.* 107:3199-3203.

**7) Erciyas, F., Taneli, F., Arslan, B., Uslu, Y. (2004):** Glycemic control, oxidative stress, and lipid profile in children with type 1 diabetes mellitus. Arch Med Res Vol. 35, pp. 134-140.

**8) Gonçalves LH, Silva MV, Duarte RC, Dusse LM, Fernandes AP, Bosco AA, Gomes KB, Carvalho MD (2014):** Acetylsalicylic acid therapy: influence of metformin use and other variables on urinary 11-dehydrothromboxane B2 levels. Clin Chim Acta. 15;429:76-78.

**9) Kaneto, H., Katakami, N., Kawamori, D., Miyatsuka, T., Sakamoto, K., Matsuoka, T.A., Matsuhisa, M. & Yamasaki, Y. (2007**): Involvement of oxidative stress in the pathogenesis of diabetes. Antioxid Redox Signal Vol. 9, pp. 355-366.

**10) Levy D (2011)**: Macrovascular complication.oxoford DiabetesLibrary: Type 1 Diabetes:105.

**11) Maahs, D, Wadwa R, Bishop F, Daniels S, Rewers M, Klingensmith GJ (2008):** Dyslipidemia in youth with diabetes: to treat or not to treat? J Pediatr. 153(4): 458-465.

**12)** [**Petitti DB**](http://www.ncbi.nlm.nih.gov/pubmed?term=Petitti%20DB%5BAuthor%5D&cauthor=true&cauthor_uid=17283301)**,** [**Imperatore G**](http://www.ncbi.nlm.nih.gov/pubmed?term=Imperatore%20G%5BAuthor%5D&cauthor=true&cauthor_uid=17283301)**,** [**Palla SL**](http://www.ncbi.nlm.nih.gov/pubmed?term=Palla%20SL%5BAuthor%5D&cauthor=true&cauthor_uid=17283301)**,** [**Daniels SR**](http://www.ncbi.nlm.nih.gov/pubmed?term=Daniels%20SR%5BAuthor%5D&cauthor=true&cauthor_uid=17283301)**,** [**Dolan LM**](http://www.ncbi.nlm.nih.gov/pubmed?term=Dolan%20LM%5BAuthor%5D&cauthor=true&cauthor_uid=17283301)**,** [**Kershnar AK**](http://www.ncbi.nlm.nih.gov/pubmed?term=Kershnar%20AK%5BAuthor%5D&cauthor=true&cauthor_uid=17283301)**,** [**Marcovina S**](http://www.ncbi.nlm.nih.gov/pubmed?term=Marcovina%20S%5BAuthor%5D&cauthor=true&cauthor_uid=17283301)**,** [**Pettitt DJ**](http://www.ncbi.nlm.nih.gov/pubmed?term=Pettitt%20DJ%5BAuthor%5D&cauthor=true&cauthor_uid=17283301)**,** [**Pihoker C**](http://www.ncbi.nlm.nih.gov/pubmed?term=Pihoker%20C%5BAuthor%5D&cauthor=true&cauthor_uid=17283301)**;** [**SEARCH for Diabetes in Youth Study Group**](http://www.ncbi.nlm.nih.gov/pubmed?term=SEARCH%20for%20Diabetes%20in%20Youth%20Study%20Group%5BCorporate%20Author%5D) **(2007):** Serum lipids and glucose control: the SEARCH for Diabetes in Youth study. [Arch Pediatr Adolesc Med.](http://www.ncbi.nlm.nih.gov/pubmed/17283301) 161(2):159-165.

**13) Rama N, Jerry LN (2004):** Lipid Inflammatory Mediators in Diabetic Vascular Disease. Arteriosclerosis, Thrombosis, and Vascular Biology. 24:1542-1548.

**14) Ramakrishna V, Jailkhani R (2007):** Evaluation of oxidative stress in Insulin Dependent Diabetes Mellitus (IDDM) patients Diagnostic Pathology.

**15) Shaw JE, Sicree RA and Zimmet PZ (2010):** Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract; 87(1): 4-14.

**16) Uttra,KM, Devrajani BR, Shah SZA, Devrajani T, Das T, Raza S, Naseem (2011):** Lipid Profile of Patients with Diabetes mellitus (A Multidisciplinary Study). World Applied Sciences Journal 12 (9): 1382-1384.

**17) Varashree BS, Bhat GP (2011):** Correlation of Lipid Peroxidation with Glycated Haemoglobin Levels in Diabetes Mellitus. Online Journal of Health and Allied Sciences 10(2):11.

**18) Viviana C, Fabiana M, Samuele S, Federico G, Isabella S, Fabrizio V, Luca D, Anna G, Elena T, Donatella C (2010):** Simultaneous quantification of 8-iso-prostaglandin-f2α and 11-dehydro thromboxane b2 in human urine by liquid chromatography–tandem mass spectrometry. Analytical Biochemistry 397:168-174.

**19) Wegner M, Pioruńska-Stolzmann M, Araszkiewicz A, Zozulińska-Ziołkiewicz D, Wierusz-Wysocka B (2011):** Evaluation of paraoxonase 1 arylesterase activity and lipid peroxide levels in patients with type 1 diabetes. Pol Arch Med Wewn 121:448-455.