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# Determinants of atherosclerosis in children and adolescents with diabetes type 1

## Ocena stopnia zaawansowania miażdżycy u dzieci i młodzieży z cukrzycą typu 1

***Sandra M. Stanković[1], Saša R. Živić[1], Ljiljana Šaranac[1], Vesna Cvetković[1], Milica Pešić[2], Karin Vasić[1], Miodrag Stanković[3], Aleksandra Topalović[4], Tatjana Cvetković[5]***

*[1]Clinic for Children's Internal Diseases, Clinical Centre, University of Nis, Nis, Serbia*
*[2]Department of Endocrinology, Clinic for Internal Medicine, Clinical Centre, University of Nis, Nis, Serbia*
*[3]Department of Child and Adolescent Psychiatry, Clinical Centre, University of Nis, Nis, Serbia*
*[4]Department of Medical Statistics, Faculty of Medicine, University of Nis, Nis, Serbia*
*[5]Biochemistry Institute, Faculty of Medicine, University of Nis, Nis, Serbia*

### Abstract

**Introduction:** To evaluate the degree of atherosclerosis in children and adolescents with type 1 diabetes and its correlation with risk factors, traditional and other, such as anti-oxidative capacity of circulating blood and level of lipid peroxidation.
**Material and methods:** Forty children and adolescents with type 1 diabetes with mean age 13.7 years were compared with 20 age- and sex-matched healthy control subjects. Association of carotid artery intima-media thickness (cIMT) with different risk factors measured in children with type 1 diabetes was evaluated.
**Results:** Mean carotid IMT was higher in subjects with diabetes ($p < 0.01$) and was strongly associated with total cholesterol with an odds ratio of 4.08 ($p = 0.016$), LDL-cholesterol with an odds ratio of 2.78 ($p = 0.037$), length of disease with an odds ratio of 1.87 ($p = 0.007$) and positive family history (first- and second-degree relatives) of diabetes and early CVD (heart attack and/or stroke before the age of 60 years) with an odds ratio of 6.8 ($p = 0.007$).
**Conclusions:** We found significantly increased cIMT in the diabetic patients compared to the healthy control subjects. Risk factors for the development of atherosclerosis included higher total and LDL-cholesterol, higher systolic blood pressure, positive family history of diabetes and early CVD and longer diabetes duration. In spite of the documented increased oxidative stress, we failed to establish a correlation between the oxidative stress parameters and cIMT values. **(Endokrynol Pol 2012; 63 (6): 414–419)**

***Key words:*** *determinants, atherosclerosis, children, diabetes type 1*

### Streszczenie

**Wstęp:** Ocena stopnia zaawansowania miażdżycy u dzieci i młodzieży z cukrzycą typu 1 oraz jego korelacja z czynnikami ryzyka, tradycyjnymi i innymi, na przykład potencjałem antyoksydacyjnym krwi krążącej i poziomem peroksydacji lipidów.
**Materiał i metody:** Porównano 40 dzieci i nastolatków z cukrzycą typu 1, średnia wieku 13,7 roku, z 20 zdrowymi osobnikami stanowiącymi grupę kontrolną odpowiednio dobranymi pod względem wieku i płci. Oceniano związek pomiędzy grubością kompleksu błony wewnętrznej i środkowej tętnic szyjnych (cIMT) a różnymi czynnikami ryzyka ocenianymi ilościowo u dzieci z cukrzycą typu 1.
**Wyniki:** Średnia wartość cIMT była większa u pacjentów z cukrzycą ($p < 0,01$) i wykazywała silny związek ze stężeniem cholesterolu całkowitego [iloraz szans (OR) 4,08; $p = 0,016$], cholesterolem frakcji LDL (OR 2,77; $p = 0,037$), czasem trwania choroby (OR 1,87; $p = 0,007$) i dodatnim wywiadem rodzinnym (krewni pierwszego i drugiego stopnia) w kierunku cukrzycy i wczesnego występowania chorób układu krążenia (zawału serca i/lub udaru mózgu przed 60. rokiem życia) (OR 6,8; $p = 0,007$).
**Wnioski:** Stwierdzono znamiennie podwyższoną wartość cIMT u pacjentów z cukrzycą w porównaniu ze zdrowymi osobnikami z grupy kontrolnej. Czynniki ryzyka rozwoju miażdżycy obejmowały podwyższone stężenie cholesterolu całkowitego i cholesterolu frakcji LDL, podwyższone skurczowe ciśnienie tętnicze, dodatni wywiad rodzinny w kierunku cukrzycy i wczesnego występowania chorób układu krążenia oraz dłuższy czas trwania cukrzycy. Pomimo udokumentowanego wzmożenia stresu oksydacyjnego nie udało się ustalić korelacji pomiędzy parametrami stresu oksydacyjnego a wartościami cIMT. **(Endokrynol Pol 2012; 63 (6): 414–419)**

***Słowa kluczowe:*** *determinanty, miażdżyca, dzieci, cukrzyca typu 1*

## Introduction

Diabetes mellitus type 1 remains the major endocrine health problem in childhood in our country, unfortunately, with growing incidence [1, 2]. Despite the progress made over the past decades in the treatment of children and adolescents with diabetes type 1, these patients still have a several fold greater risk for development of coronary, cerebrovascular and peripheral arterial diseases during their young adulthood [3–5].

In the background of these conditions lies a more aggressive process of atherosclerosis to which these

Sandra Stanković M.D., Bul. Dr Zorana Djindjica 48a, 18000Nis, Serbia, tel: +38 16 239 32 25, e-mail: stankovic_sandra@yahoo.com

patients are prone. Hyperglycaemia and increased oxidative stress play pivotal roles in the early stages of atherogenesis in diabetes, including impairment of endothelial function and the formation of fatty streaks and plaques.

Although our understanding of who is at risk for macrovascular disease in type 1 diabetes is still far from complete, patients with poor glycoregulation [6], longer duration of diabetes [7], early onset of the disease [8] accompanied by standard cardiovascular risk factors, including a family history of cardiovascular disease and type 2 diabetes, should be considered at increased risk.

Furthermore, oxidative stress as well as defects in antioxidant defence systems are recognised as causative factors for the development of major diabetic complications [9].

Common carotid artery intima-media thickness (cIMT) measured by high resolution B-mode ultrasonography is a non-invasive marker of subclinical atherosclerosis that has been used widely in these patients to assess vascular health [10]. However, information on the association of cIMT with different risk factors measured in children with type 1 diabetes is still limited.

Therefore, we evaluated the determinants of atherosclerosis, traditional and other, such as anti-oxidative capacity of circulating blood and level of lipid peroxidation and their connection with cIMT in children and adolescents with type 1 diabetes.

## Material and methods

### *Patients*

Forty children with diabetes mellitus type 1 and 20 age- and sex-matched control subjects were enrolled in the study. The diagnosis of diabetes mellitus was based on the current criteria of the American Diabetes Association. All patients were recruited consecutively during their regular three-month visits as outpatients at a tertiary health care centre (Children's Hospital Niš, Division of Endocrinology). Medical records were available for all patients (data on HbA1c and insulin dosage as well as on diabetic complications) for the entire follow-up period. Subjects were excluded if they had evidence or history of clinically relevant systemic disease. All patients were free of any microvascular complication of diabetes (retinopathy, nephropathy or neuropathy). The mean HbA1c was calculated as the arithmetic mean value of the last three HbA1c levels measured during the regular follow-up visits at the outpatient department. Family history (first- and second-degree relatives) of diabetes type 2 and early CVD (heart attack and/or stroke before the age of 60 years) was determined by questionnaire. Written informed consent was obtained for all participants from their legal guardians and the study protocol was approved by the local ethics committee. The mean age of patients was 13.9 ± 2.8 years. The control group comprised 20 healthy subjects: 13 girls and seven boys, average age 13.1 years.

### *Methods*

**Blood pressure measurements**

Blood pressure was obtained using a conventional oscillatory measurement system positioned at the right upper arm. The size of the cuff was chosen depending on the patient's arm circumference, with the cuff bladder covering at least 40% up to a maximum of 100% of the arm circumference.

**Laboratory procedures**

Blood samples were taken during the patients' follow-up visit. Fasting HbA1c, triglycerides, total cholesterol, high density (HDL) and low density cholesterol were measured by standard laboratory methods.

**Oxidative stress parameters**

Thiobarbituric acid reacting substances (TBARS) content, a measure of lipid peroxidation, was assayed in plasma and red blood cells according to the methods of Andreeva [11] and Jejin [12].

The **catalase activity** was determined by the spectrophotometric method, based on the ability of hydrogen peroxide to form a stable stained complex with molybdenum salts [13]. An improved method for the determination of total serum **SH groups** was based on the formation of colour product, monitored at 412 nm after addition of Ellman reagent (5, 5′-dithiobis-2-nitrobenzoic acid) [14].

**Ultrasonography**

All ultrasound studies were done according to a standardised scanning protocol for the right and left common carotid arteries [15]. High resolution carotid ultrasound studies were performed (Acuson, Siemens) with a 12 MHz transducer. Before beginning the ultrasound imaging, subjects lay quietly on a bed. The study protocol involved scanning of the far wall of the right and left common carotid arteries in the distal 1.0–1.5 cm. In each examination, different scanning angles (anterior, latero-posterior) were adopted to record the greatest intima-media thickness. Three determinations of the right and the left carotid artery were performed and these three determinations were averaged. Furthermore, the right and the left carotid artery measurements were averaged and used for analysis (mean cIMT). The examiners were blinded to the subject. Ten patients presented twice for the determination of intraobserver variability within seven weeks and the cIMT was measured without knowledge of the previous values. The intraobserver variability was ≤ 5%.

**Table I.** *Anthropometric, clinical, biochemical and ultrasonic characteristics*
**Tabela I.** *Charakterystyka antropometryczna, kliniczna, biochemiczna i ultrasonograficzna*

| | Study group (n = 40) | Control group (n = 20) | p |
|---|---|---|---|
| Age | 13.86 ± 2.73 | 13.18 ± 2.58 | 0.358 |
| Sex (male/female) | 27/13 | 11/9 | 0.507 |
| Weight [kg] | 47.33 ± 13.66 | 42.20 ± 10.06 | 0.142 |
| Height [cm] | 156.42 ± 14.93 | 155.88 ± 14.39 | 0.894 |
| BMI | 18.92 ± 3.28 | 17.15 ± 2.36 | 0.036* |
| Smoking (%) | 7,5% | 5% | 0,644 |
| Total cholesterol [mg/dL] | 4.53 ± 0.80 | 4.41 ± 0.63 | 0.561 |
| LDL-cholesterol [mg/dL] | 3.16 ± 0.86 | 3.13 ± 0.64 | 0.891 |
| HDL-cholesterol [mg/dL] | 0.96 ± 0.36 | 0.95 ± 0.23 | 0.910 |
| Triglycerides [mg/dL] | 0.82 ± 0.66 | 0.79 ± 0.72 | 0.872 |
| HbA1c | 9.23 ± 1.63 | 5.1 ± 0.6 | < 0.001*** |
| MDAs [$\mu$mol/L][1] | 29.12 ± 16.16 | 7.6 ± 1.56 | < 0.001*** |
| MDAer [$\mu$mol/L)][2] | 13.03 ± 3.61 | 4.17 ± 0.68 | < 0.001*** |
| SH [$\mu$mol/L][3] | 347.52 ± 223.39 | 393.65 ± 114.94 | 0.390 |
| Catalase | 136.14 ± 36.46 | 129.97 ± 41.29 | 0.557 |
| Mean combined cIMT [mm][4] | 0.45 ± 0.06 | 0.40 ± 0.06 | < 0.003** |

*p < 0.05, **p < 0.01, ***p < 0.001; [1]MDAS — malondialdehyde in sera; [2]MDAer — malondialdehyde in red blood cells; [3]SH — sulphhydryl group; [4]cIMT — carotid artery intima-media thickness

### *Statistical analysis*

Statistical analyses were performed using SPSS 16.0. Values are expressed as mean ± SD. Comparisons between subjects with diabetes mellitus type 1 and controls were made using paired Student's t-tests and chi-square analysis for continuous and categorical variables, respectively. To examine risk factors, univariate logistic regression was used. A p value of < 0.05 was considered statistically significant.

## Results

The clinical characteristics of the participants in the present study are shown in Table I. The groups (diabetic patients and healthy control subjects) were similar regarding sex and age. However, a significant difference was observed between the groups regarding BMI with higher values among the diabetic patients (p < 0.036).

Considering lipid profile, there was no significant difference between patients and the control group. In the diabetic group, mean HgbA1c was higher 9.23 ± 1.63 % (p < 0.01).

Oxidative stress was assesed by measuring the level of malondialdehyde (MDA), a marker of lipid peroxidation in serum and erythrocites. We found a significantly increased level of lipid peroxidation in diabetic patients in serum 29.12 ± 16.16 $\mu$mol/L *v.* 7.6 ± 1.56 $\mu$mol/L (p < 0.001), and in erythrocites 13.03 ± 3.61 $\mu$mol/L *v.* 4.17 ± 0.68 (p < 0.001). In spite of high lipid peroxidation in the diabetic group, the activity of antioxidative enzymes catalase and tiol groups were modest, with no significant difference within groups. cIMT was significantly higher in the diabetic than in the control group 0.45 ± 0.06 *v.* 0.40 ± 0.06 (p < 0.003).

Furthermore, we divided diabetic patients into two groups: the first group with cIMT below the 95$^{th}$ percentile and the second group with cIMT above the 95$^{th}$ percentile, according to proposed normative values for European children [14] (Table II). We compared their clinical and metabolic caracteristics. The group with cIMT above the 95$^{th}$ percentile was significantly older 14.77 ± 2.38 years *v.* 12.61 ± 2.75 years (p < 0.009), taller 162.00 ± 11.16 cm *v.* 148.75 ± ± 16.33 cm (p < 0.002), had greater body weight 53.20 ± 11.22 kg *v.* 39.25 ± 11.44 kg (p < 0.006), higher BMI (p < 0.003) and had longer disease duration 6.94 ± ± 4.70 years *v.* 3.22 ± 2.68 (p < 0.001) years compared to the other group.

No significant difference was found in systolic and diastolic blood pressure, age at onset of disease, insulin dose, and level of HgbA1c between these two groups. Fourteen patients with cIMT above the 95$^{th}$ percentile, and only two patients from the other group, were on intensive insulin treatment.

**Table II.** *Clinical characteristics of patients with cIMT < P95th and cIMT > P95th*
**Tabela II.** *Charakterystyka kliniczna pacjentów z cIMT poniżej 95. percentyla i cIMT powyżej 95. percentyla*

| | cIMT < P95th (n = 17) | cIMT > P95th (n = 23) | p |
|---|---|---|---|
| Age (years) | 12.61 ± 2.75 | 14.77 ± 2.38 | 0.009* |
| Sex (male/female) | 6/11 | 7/16 | 0.986 |
| Weight [kg] | 39.25 ± 11.44 | 53.20 ± 11.22 | 0.006* |
| Height [cm] | 148.75 ± 16.33 | 162.00 ± 11.16 | 0.002* |
| BMI | 17.32 ± 2.18 | 20.09 ± 3.47 | 0.003* |
| Diastolic blood pressure [mm Hg] | 66.43 ± 6.63 | 66.00 ± 7.37 | 0.079 |
| Systolic blood pressure [mm Hg] | 107.50 ± 7.78 | 111.33 ± 5.16 | 0.729 |
| Insulin dose I/kg | 0.74 ± 0.28 | 0.96 ± 0.44 | 0.079 |
| Conventional/intensive insulin therapy (n) | 15/2 | 9/14 | 0.004* |
| Age at onset (years) | 8.82 ± 3.96 | 7.94 ± 3.38 | 0.448 |
| Diabetes duration (years) | 3.22 ± 2.68 | 6.94 ± 4.70 | 0.001** |
| Total cholesterol [mg/dL] | 4.15 ± 0.58 | 4.82 ± 0.83 | 0.007* |
| LDL-cholesterol [mg/dL] | 2.86 ± 0.68 | 3.48 ± 0.93 | 0.025* |
| HDL-cholesterol [mg/dL] | 0.95 ± 0.28 | 0.97 ± 0.40 | 0.857 |
| Triglycerides [mg/dL] | 0.76 ± 0.76 | 0.84 ± 0.57 | 0.704 |
| Hba1c | 8.99 ± 1.19 | 9.42 ± 1.90 | 0.315 |
| MDAs [μmol/L] | 22.62 ± 15.72 | 35.19 ± 14.53 | 0.246 |
| MDAer [μmol/L] | 12.68 ± 2.65 | 13.36 ± 4.39 | 0.544 |
| SH [μmol/L] | 392.86 ± 229.21 | 305.20 ± 216.91 | 0.124 |
| Catalase | 138.58 ± 31.09 | 133.86 ± 41.84 | 0.737 |

*p < 0.05, **p < 0.01

**Table III.** *Risk factors for intima-media thickness (univariate logistic regression)*
**Tabela III.** *Czynniki ryzyka dla zwiększonej grubości kompleksu błony wewnętrznej i środkowej (jednoczynnikowa regresja logistyczna)*

| Independent variable | OR | 95% CI | P |
|---|---|---|---|
| Total cholesterol | 4.08 | 1.31–12.74 | 0.016* |
| LDL-cholesterol [mg/dL] | 2.78 | 1.06–7.28 | 0.037* |
| Systolic blood pressure [mm Hg] | 1.11 | 0.99–1.24 | 0.081* |
| Duration of disease (years) | 1.87 | 1.19–2.95 | 0.007* |
| Positive family history of diabetes type 2 and early CVD | 6.8 | 1.68–27.52 | 0.007* |

OR — odds ratio; 95% CI — 95% confidence interval

Patients with increased cIMT had significantly higher levels of total ($p < 0.007$) and LDL cholesterol ($p < 0.025$).

Considering oxidative stress, we found a nonsignificantly higher level of lipid peroxidation, and nonsignificantly lower activity of catalase and thiol groups in the group with cIMT above the 95th percentile.

Univariate logistic regression showed that the increased cIMT in 40 patients was significantly associated with total cholesterol with an odds ratio of 4.08 ($p = 0.016$), LDL-cholesterol with an odds ratio of 2.78 ($p = 0.037$), length of disease with an odds ratio of 1.87 ($p = 0.007$) and positive family history (first- and second-degree relatives) of diabetes type 2 and early CVD (heart attack and/or stroke before the age of 60 years) with an odds ratio of 6.8 ($p = 0.007$) (Table III).

## Discussion

Several researchers have conducted studies on cIMT in paediatric patients with diabetes mellitus type 1 [16–20]. Like most cited studies on the subject [18, 21], the present study revealed a significantly increased intima-media thickness in a group of children and adolescents with diabetes mellitus type 1.

The diabetic patients in this study had higher BMI than the control subjects, as shown in previous studies. This is often explained by overinsulinisation during intensive insulin treatment, frequent hypoglycaemic episodes treated by additional food intake, and nutritional liberation leading to increased energy intake [22].

A direct association between age, height, weight, BMI and cIMT was found in this study. Although cIMT increases with age in adults, this relationship has not been clearly demonstrated in children and adolescents [19, 23]. It was shown in previous studies that cIMT increases slightly with age but is more closely related to height than to age [16].

According to current ISPAD guidelines of quality of glycaemic control, our patients had poor metabolic control with mean HgbA1c of 9.23 ± 1.63%. Consistent with previous studies, we were not able to establish a direct association between the HbA1c and the values of IMT. Data from the literature indicates, however, that in contrast to the functional impairment of the endothelium, structural changes do not correlate to a single parameter such as the HbA1c at a young age [8].

In the diabetic group as a whole, in spite of poor glycaemic control, the mean values of total, HDL and LDL cholesterol, triglycerides, were not significantly higher than in the control subjects. But in the group with elevated cIMT, total and LDL cholesterol were significantly higher than in the group with smaller cIMT. Our results are comparable with some previous studies [19, 24].

Lipids, the substrate under greatest attack by free radicals, have been extensively studied, and in diabetic patients are of special interest because their hyperlipidaemia is considered to be a significant risk factor for the development of vascular complications [25, 26]. Peroxidation of fatty acids produces aldehydes such as thiobarbituric acid reacting substances (TBARS). The most commonly measured TBARS is malondialdehyde [27]. We documented significantly increased lipid peroxidation in the sera and red blood cells of type 1 diabetes patients.

Catalase is one of the most potent and widely distributed antioxidant enzymes. Catalase has a role in eliminating hydrogen peroxide before it has a chance to react with ferro ions and form potent free oxygen radicals, such as hydroxil radicals. Thiol groups such as glutathion, cystein and homocystein (all contain SH groups and are referred to as sulphhydryl groups) represent another important antioxidant defence system. Glutathion is oxidated by lipid peroxides under the influence of glutathion peroxidase yielding glutathion disulphide, indicating that glutathion may serve a purpose in detoxifying lipid peroxides. Relatively low activity of catalase and thiol groups in the condition of significantly increased lipid peroxidation in our study could be a sign of impairment of antioxidative activity in diabetic patients. We have documented that oxidative stress takes place early in type 1 diabetes through increased lipid peroxidation, but we failed to establish a direct correletion between oxidative stress and cIMT.

In our study, no significant correlation between cIMT and age at onset, insulin dosage or intensive insulin treatment was found.

## Conclusions

In conclusion, we were able to show significantly increased cIMT in 23 patients out of a total of 40 children and adolescents with type 1 diabetes mellitus. Risk factors for the development of atherosclerosis included higher total and LDL-cholesterol, higher systolic blood pressure, positive family history of diabetes and early CVD and longer diabetes duration. In spite of the high level of lipid peroxidation and documented poor glycaemic control, we were not able to establish a direct correlation between the HbA1c and lipid peroxidation with the values of cIMT. Measuring cIMT is useful tool in determining early atherosclerosis that can give us information as to who is at increased risk of developing macrovascular complications on an individual basis. Lowering cardiovascular risk in this high-risk group of children must be warranted [28].

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