

## IMPORTANCE OF GOOD GLUCOREGULATION IN PREVENTION OF ATHEROSCLEROSIS IN CHILDREN AND ADOLESCENTS WITH DIABETS MELLITUS TYPE 1

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Patients with diabetes mellitus type 1 are at significant risk of cardiovascular disease. Non-invasive ultrasound measurement of carotid intima-media thickness is used for evaluation of subclinical atherosclerosis *in vivo*.

The aim of the paper was to evaluate the degree of atherosclerosis and its correlation with glucoregulation in children with type 1 diabetes.

Carotid artery intima-media thickness (cIMT) was measured in 41 subjects with type 1 diabetes (mean [SD] age = 13,8 (3,2) years) and 20 control subjects (13,1 (2,8) years). Degree of glycemic control was determined using the levels of average HbA1c and average postprandial glucose.

Mean carotid IMT was higher in subjects with diabetes (P=0.01). Mean carotid IMT was significantly higher in subjects with suboptimal and poor glucoregulation than in subjects with optimal glycemic control and healthy subjects. Postprandial glucose level was significantly lower in the group with optimal glucoregulation than in the groups with suboptimal (P=0,05) and poor glucoregulation (P=0,01); however, statistically significant correlation between the level of postprandial glucose and cIMT was not registered.

cIMT measurement enables the identification of patients at increased risk of cardiovascular disease on an individual basis, as well as appropriate modification of patient treatment. *Acta Medica Medianae* 2008;47(2):5-9.

**Key words:** diabetes, atherosclerosis, glucoregulation

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### Introduction

Although complications of atherosclerosis, such as coronary disease, stroke and periphery vascular disease usually occur in middle-aged or older persons, autopsy studies showed that atherosclerotic vascular process might begin as early as childhood and accelerate if certain risk factors were present (1,2).

Persons having diabetes mellitus type 1 have two to four times greater risk of developing atherosclerotic vascular diseases (3-5). Diabetes-associated macrovasculopathy is a consequence of structural and functional changes in blood vessels that occur due to numerous pathophysi-

ological disorders. Among them hyperglycemia occupies an important position, leading to non-enzyme glycolisation of protein, increased oxidative stress, lipid peroxidation and activation of cell signaling pathways cascades. These disorders cause endothelial dysfunction with balance disorder between vasoconstrictors and vasodilators, procoagulant and anticoagulant mediators, as well as between growth-stimulating and growth inhibiting factors (6-8).

Recently, postprandial serum glucose concentration has often been mentioned as one of the main predictors of vascular disorders in patients with diabetes mellitus (9).

A noninvasive ultrasound measurement of carotid artery posterior wall intima-media thickness (cIMT) is used for examination of subclinical atherosclerosis *in vivo* (10,11). cIMT is a marker of generalized atherosclerosis that correlates with the degree of coronary disease and represents a strong predictor of future possible cardiovascular events in adults (12,13). There are numerous papers suggesting that obese children and children with family history of hypercholesterolemia, as well as adults suffering from diabetes mellitus type 1, have increased cIMT (14, 15). Examinations on intima-media thickness in

children and adolescents with diabetes mellitus 1 produced contradictory results (16-18).

During 2004 an investigation was conducted and on its basis referential values of carotid artery intima-media in healthy children were suggested. They are in the range up to 0,45 mm for children under 10 years of age, and for children older than 10 and younger than 18 up to 0,55 mm (19).

### Material and methods

The investigation was conducted in the Endocrinology Department of the Children's Internal Clinic in Nis and in the Institute of Biochemical of the Faculty of Medicine in Nis.

The investigation enrolled 61 subjects: 41 subjects with diabetes mellitus type 1 and 20 healthy children.

The subjects with diabetes mellitus (n=41) were recruited during their regular quarterly check-ups in Daily hospital of the Children's Internal Clinic. Average age of the subjects was 13,8 years. The youngest patient was 6,5 years old, and the oldest was 19,5 years old. There were 28 girls and 13 boys.

Diagnosis of this disease is established in accordance with National Diabetes Data Group recommendations, accepted by the World Health Organization.

The control group comprised 20 healthy children: 13 girls and 7 boys, average age 13,1 years. Age range was from 7,5 to 18,7 years.

Diagnosis is established in accordance with National Diabetes Data Group recommendations, accepted by the World Health Organization. There were no signs of chronic diabetic microvascular complications (retinopathy, microalbuminuria, neuropathy) in any of the subjects.

Children and adolescents with some other systemic disease (growth hormone deficit, hypothyroidism, celiac disease) or an acute infection were excluded from the study.

During the evaluation of gluco-regulation impact on examined parameters, the subjects were put into three groups on the basis of ISPAD recommendations (20) on gluco-regulation quality:

- Group A (n=6) with optimal gluco-regulation (HbA1c  $\leq$ 7,5%),
- Group B (n=14) with suboptimal gluco-regulation (HbA1c 7,6% - 9,00%),
- Group C (n=21) with poor gluco-regulation (HbA1c >9,01).

Clinical status was determined by general pediatric examination. Body weight and body height were measured, percentiles were determined, body mass index (BMI) was calculated according to the formula  $BMI = kg/m^2$ .

Patients were followed up for six months for adequate evaluation of their metabolic regulation. For evaluation of gluco-regulation mean values of HbA1c were used, as well as mean values of postprandial glucose concentration in serum, obtained on the basis of three sequential measurements

during the regular quarterly check-ups in Daily hospital of the Children's Internal Clinic: initially, after three months and after six months.

Glucose concentration was determined by standardized laboratory analysis in biochemical laboratory in the Children's Clinic using the apparatus Olympus AU 400.

Hemoglobin A1c was determined by chromatography with ion-exchangers on the column of the Bio Rad firm. Referential values: 4,2-5,9%.

Left and right carotid artery intima-media thickness (cIMT) was determined by high-resolution B-mode ultrasound (Hewlett Packard Image Point) with a 8 MHz probe. All the examinations were conducted by a single experienced radiologist who was unaware of metabolic condition of the patients. In all the patients intima-media thickness was measured at the posterior wall of carotid artery by predetermined standards for measurement (10,11). Ultrasound examination was performed in the lying position. After ultrasound identification of carotid artery, its posterior wall was focused 1-2 cm proximal to the carotid bulb and cIMT was measured at two angles: anterior oblique and lateral. Space thickness between the leading edge of the lumen-intima interface and the leading edge of the media-adventitia interface was measured on B mode. Three measurements of cIMT were done and an arithmetic mean value of these three measurements was shown as the result.

Ten patients were examined twice a month to determine reproducibility of the method. Difference in cIMT thickness was less than 10% (average 5%).

Statistic data processing was done in Sigma Pet programme. For statistic data processing mean, median and standard deviation were used in testing significance of differences: Student's t-test for paired samples, non-parametric Mann-Whitney test, Pearson's chi-square ( $\chi^2$ ) test. Correlations were analysed using Pearson's linear correlation test. A level quoted for all significances was  $p < 0,05$ . A multiple linear regression analysis was carried out to test multivariate correlation between IMT as a dependant variable and cardiovascular risk factors as independent variables.

### The aim of the study

The aim of the study was to evaluate carotid artery wall intima-media thickness as a marker of preclinical atherosclerosis in children and adolescents affected by diabetes mellitus type 1 and correlation of the degree of preclinical atherosclerosis with gluco-regulation quality.

### Results

There were 28 girls and 13 boys in the group of children and adolescents affected by diabetes mellitus type 1. The control group comprised 13 girls and 7 boys. Distribution according to sex was equable in the examined groups (Table 1).

Table 1 – distribution according to sex

Sex	Control		Patients	
	n	(%)	n	(%)
Boys	7	35	13	33
Girls	13	65	28	67
Total	20	100	41	100

\*no statistic significance

Mean values with standard deviations, as well as maximum and minimum age values and BMI index values in the examined groups are given in Table 2.

In the group of children and adolescents affected by diabetes mellitus type 1, average age was 13,8 years, the youngest patient was 6,5 years old, and the oldest 19,5 years old. Average age in the control group was 13,1 years. Age range was between 7,5 to 18,7 years. Mean values of BMI index in children affected by diabetes were slightly higher than mean BMI index in the control group, but with no statistical significance.

Table 2 – Anthropometric characteristics of the subjects

	Patients		Control	
	X±SD	Max-min	X±SD	Max-min
Age	13,8±3,2	19,5-6,5	13,1±2,8	18,7-7,5
BMI	18,9±3,3	27,5-14	17,2±2,4	22,3-13,4

\* no statistic significance

Table 3 shows mean values with standard deviations of HbA1c in subjects A, B and C. The results show statistically significant difference on multiple levels. The subjects with optimal glucoregulation have statistically higher values of HbA1c in comparison to the control group ( $p < 0,001$ ). The subjects with suboptimal glucoregulation have statistically higher values of HbA1c in comparison to the control group and statistically significantly lower value of HbA1c compared to the group of subjects with poor glucoregulation. The subjects have significantly higher values of HbA1c in comparison to the control group.

Table 3. Values of HbA1c

Group	HbA1c (%)
Control	5,1±0,6
A	6,9±0,4 <sup>a,b,c</sup>
B	8,5±0,5 <sup>d,e</sup>
C	10,4±1,3 <sup>f</sup>

a-  $p < 0,001$  vs. Control, b-  $p < 0,001$  vs. group B, c-  $p < 0,001$  vs. Group C, d-  $p < 0,01$  vs. Control, e-  $p < 0,01$  vs. Group C, f-  $p < 0,001$  vs. Control

Mean values with standard deviations, minimum and maximum glucose concentrations 1,5 h after a meal are given in Table 4. There is considerably lower postprandial serum glucose concentration in subjects with optimal glucoregulation in comparison

to the subjects with suboptimal and poor glucoregulation (Table 4).

Table 4. Glucose concentration in serum 1,5 h after a meal

Group	Postprandial glucose (mmol/l)	Max-min
A	9,7±2,2 <sup>a,b</sup>	14,2-9,0
B	11,7±1,7	21,3-5,2
C	15,1±17,8	33,1-6,5

$p < 0,05$  vs. group B, b-  $p < 0,01$  vs. group C

Table 5 – Values of cIMT

Group	cIMT (mm)
Control	0,50±0,04
A	0,53±0,07
B	0,57±0,05 <sup>a</sup>
C	0,54±0,07 <sup>b</sup>

a,b-  $p < 0,01$  vs. control

Table 5 shows measured mean values with standard deviations of carotid intima-media thickness (cIMT) in the subjects. Our study has shown statistically significantly higher cIMT in subjects from the group with suboptimal and optimal glucoregulation in comparison to the control group ( $p < 0,01$  right and left cIMT). The subjects from the group with optimal glucoregulation did not present statistically significant difference in comparison to cIMT thickness in the control group.

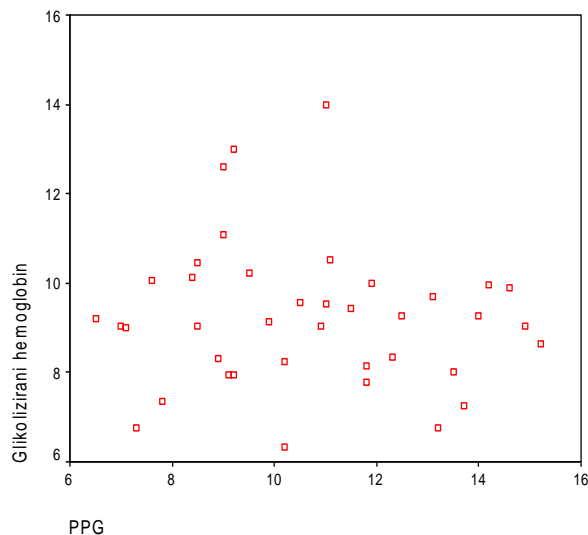
Table 6 – Correlation coefficients

	HbA1c (%)	kgp	cIMT
HbA1c (%)	/	0,8	0,8
kgp	0,3	/	0,9
cIMT	0,8	0,9	/

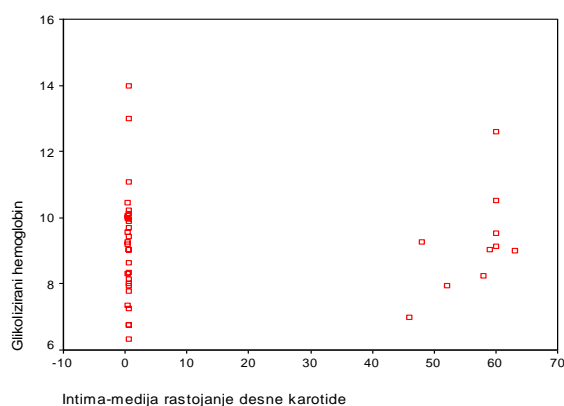
\*kgp-postprandial glucose concentration

\*no statistical significance

Table 6 shows correlation among examined parameters. The correlations were not found to be statistically significant.



Graph 1 – Correlation between glycosylated hemoglobin and postprandial glucose concentration



Graph 2 – Correlation between glycosylated hemoglobin and the right cIMT

## Discussion

Diabetes mellitus type 1 is a significant risk factor for accelerated and early onset of atherosclerosis. Numerous studies have shown that adult patients with diabetes mellitus type 1 had increased cIMT (13,21,22). Examinations of intima-media thickness in children and adolescents with diabetes mellitus type 1 produced contradictory results (16-19).

The largest cIMT study conducted in the USA in 142 children and adolescents with diabetes mellitus type 1 showed significant increase of cIMT in children and adolescents with diabetes mellitus type 1 in comparison to the control group of healthy children (23). Also, Nieuwdorp et al. (24) and Dalla Pozza et al. (25) showed increased cIMT in children with diabetes mellitus type 1. Contrary to these results, Yavuz T. (16) in his study that enrolled 52 children with diabetes did not find significant impact of diabetes on cIMT thickness. In our study the patients affected by diabetes mellitus type 1 have significant increase of cIMT in comparison to the control group.

The results of our investigation show significantly higher values of serum glucose concentrations in the groups of patients with diabetes in comparison to the control group, and it is in accordance with the results of other studies (24,25). In our study only 6 subjects, that is 14,5%, had

optimal gluco-regulation, 35,6 suboptimal gluco-regulation, and 50 poor gluco-regulation.

Obtained results are in accordance with Hvidore study that was conducted in the period 1995–1998 and enrolled 2101 adolescents, aged 10 - 18 years, representing 17 countries from Europe, Japan and North America (26). This multicenter study of pediatric diabetes was designed to evaluate the current level of metabolic control in children and adolescents with insulin-dependent diabetes mellitus, frequency of severe hypoglycemias and body weight gain. The study showed unsatisfactory level of metabolic control in many subjects, despite the insulin regime (HbA1c <7,6% only in 17,5%). Such poor gluco-regulation could be explained by already mentioned problems that accompany pediatric diabetes, poor socio-economic living conditions, low health-care education, and, on the other side, insufficiently developed endocrinology services in our region.

Our results show importance of good gluco-regulation. The group of children with optimal gluco-regulation did not have statistically significantly higher values of cIMT in comparison to the control one, contrary to the groups of children with suboptimal and poor gluco-regulation, that had statistically significantly increased cIMT compared to the control group. Obtained results are in accordance with data gathered in a large DCCT study (27), as well as with some other authors' studies (25), but are contrary to the results of Nieuwdorp et al. (24) who did not find connection of HbA1c and cIMT levels.

Importance of good regulation is presented and shown in the case of a boy with poor control of serum glucose concentration whose cIMT was increased to the level of a 60 year old man. An intensive insulin therapy and good regulation of serum glucose concentration led to significant regression of cIMT within 41 months (28,29).

## Conclusion

cIMT measurement enables the identification of patients at increased risk of cardiovascular disease on an individual basis, as well as appropriate modification of patient treatment. Patients at increased risk must be treated more aggressively, by more intensive insulin treatment regimen, with strict gluco-regulation and reduction of the effects of all known atherogenic risk factors.

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## **ZNAČAJ DOBRE GLIKOREGULACIJE U PREVENCIJI ATEROSKLEROZE KOD DECE I ADOLESCENATA OBOLELIH OD DIJABETES MELITUSA TIP 1**

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Osobe sa dijabetes melitusom tip 1 imaju značajan rizik za razvoj kardiovaskularne bolesti. Neinvazivno ultrazvučno merenje debljine intime-medije u zadnjem zidu arterije karotis komunis (cIMT) koristi se za ispitivanje subkličičke ateroskleroze *in vivo*.

Cilj našeg istraživanja bio je evaluacija cIMT kao markera pretkličičke ateroskleroze kod dece i adolescenata obolelih od dijabetes melitusa tip 1 i povezanost stepena pretkličičke ateroskleroze sa kvalitetom glikoregulacije.

Neinvazivno ultrazvučno merenje debljine intime-medije u zadnjem zidu arterije karotis komunis (cIMT) rađeno je kod 41 ispitanika sa dijabetes melitusom tip 1 (prosečni uzrast [SD]=13,8 (3,2) godina) i kod 20 zdravih ispitanika (prosečni uzrast [SD]=13,1 (2,8) godina). Kvalitet glikoregulacije smo određivali preko prosečnih nivoa glikoziliranih hemoglobina i preko prosečnih nivoa postprandijalne glukoze.

Ispitanici oboleli od dijabetes melitusa tip 1 imali su veći cIMT u odnosu na kontrolnu grupu (P=0.01). Grupa dece sa optimalnom glikoregulacijom nije imala statistički značajno veće vrednosti cIMT u odnosu na kontrolnu, za razliku od grupe dece sa suboptimalnom (P=0.01) i lošom glikoregulacijom (P=0.01), koja su imala statistički značajno povećan cIMT u odnosu na kontrolnu grupu. Kod naših ispitanika, postprandijalne koncentracije serumske glukoze bile su statistički značajno niže kod ispitanika sa optimalnom glikoregulacijom u odnosu na grupu sa suboptimalnom (P=0.5) i lošom glikoregulacijom (P=0.01), ali nismo našli direktnu korelaciju između vrednosti prosečne postprandijalne serumske glukoze i cIMT.

Merenje cIMT omogućava identifikaciju bolesnika sa posebnim rizikom na individualnoj osnovi, a samim tim i odgovarajuću modifikaciju tretmana bolesnika. *Acta Medica Medianae* 2008;47(2): 5-9.

**Ključne reči:** dijabetes, ateroskleroza, glikoregulacija