Early macrovascular complications of carbohydrate intolerance

Thesis

Dr. Vastagh Ildikó

Semmelweis University, School of Ph.D Studies
Clinical Medicine

Tutor: Prof. Somogyi Anikó, MD, DSc
Consultant: Prof. Bereczki Dániel, MD, DSc

Referees: Prof. Kempler Péter, MD, DSc
Horváth Sándor, MD, Candidate of the Medical Science

Committee for Ph.D. examination:
Head: Prof. Rozgonyi Ferenc, MD, DSc
Members: Vásárhelyi Barna, MD, DSc
Valikovics Attila, MD, PhD

Budapest
2011

INTRODUCTION

Cardiovascular disease (CVD) continues to be the leading cause of mortality in patients with diabetes mellitus (DM). Most epidemiological and clinical-trial data have derived from studies of type 2 diabetes, in which CVD accounts for 70 percent of all deaths. The absolute risk of CVD is lower in patients with type 1 diabetes than in those with type 2 diabetes, but the relative risk of CVD still increases more than 10-fold in type 1 diabetic patients compared to non-diabetic persons of similar age. DM results in accelerated atherosclerosis.

Prospective studies have shown that increased intima-media thickness (IMT) of the common carotid artery (CCA) is a powerful predictor of coronary and cerebrovascular complications, and carotid artery IMT is a predictor of coronary artery disease in diabetes as well. Therefore, carotid IMT may provide an index of atherosclerotic vascular process that can be used to study subclinical atherosclerosis.

Arterial stiffness represents a process of vascular damage distinct from the development of atherosclerosis measured by carotid IMT. Several cardiovascular risk factors, for example age, smoking, hypertension and hypercholesterolemia are associated with arterial stiffening. Changes in elastic features of large arteries appear only after relatively long disease duration in diabetes. For example changes in both small and large artery elasticity and systemic vascular resistance were reported after longer (> 10 years) duration of type 1 diabetes.

Gestational diabetes mellitus (GDM) is defined as glucose intolerance that appears or is first recognized during pregnancy. The purpose of screening for GDM is to diagnose and treat patients as early in pregnancy as possible, thereby preventing complications possibly caused by elevated blood glucose levels in pregnancy. A number of pregnancy complications are thought to be caused by GDM, such as macrosomia, Cesarean delivery, shoulder dystocia, neonatal metabolic problems, perinatal mortality, hypertension of the pregnant and preeclampsia. GDM develops in 3-5 % of pregnant women but they have a 17-63% risk of developing type 2 diabetes mellitus (T2DM) within 5-16 years following the index pregnancy.

Women with a previous GDM (pGDM), appear to be at increased risk of CVD, such as increased IMT and stiffness of large elastic arteries and higher values of markers of endothelial dysfunction were found. Prospective studies found that women with GDM have increased cIMT compared with unaffected women. Furthermore study detected increased maternal arterial stiffness of pregnancies complicated with GDM or T2DM. On the other hand similar carotid artery functions were found during normal and diabetic pregnancy.

AIMS

1. Aims of the study of patients with type 1 diabetes mellitus

We assumed that some morphological and functional changes will appear only relatively late in the course of the disease, but still before the manifestation of clinical complications. In most reports either structural or functional features of large elastic vessels were examined, therefore the comparison of these results is difficult. On the other hand, the few studies comparing structural and functional features of arteries have controversial results. We hypothesized, that there are morphological and/or functional parameters that change earlier in the course of T1DM; therefore it may be possible to identify those patients in the preclinical stage that might have an increased risk to develop cardiovascular complications. To test this hypothesis we evaluated a set of morphological (*d*, diameter; IMT; IMCSA, intima-media cross section area) and functional (D, distensibility; C, compliance; Str, circumferential strain; SI, stiffness index β; E_{inc}, incremental elastic modulus; PWV, pulse wave velocity) features in 42 young patients with type 1 diabetes mellitus and 41 age-, gender- and BMI-matched healthy subjects. After it cardiovascular risk factors were compared to parameters of large elastic vessels.

2. Aims of the study of patients with gestational diabetes mellitus

The aim of our case control study was to assess if structural (*d*, end-diastolic diameter, cIMT; IMCSA, intima-media cross section area)

and/or functional (Δd, distension; D, distensibility; C, compliance; Str, circumferencial strain; SI, stiffness index; E_{inc}, incremental elastic modulus) characteristics of large elastic arteries indeed change in women with GDM in the 3rd trimester. We assumed that the duration of glucose intolerance during pregnancy is too short to result in significant morphological or functional changes of large elastic arteries, especially in those with well-controlled serum glucose levels.

METHODS

1. Methods in study of patients with type 1 diabetes mellitus

Subjects

42 patients (age 34 ± 10 years; 22 men and 20 women) with type 1 diabetes and without macroangiopathy (average duration 15 ± 10 years) were examined. Forty-one age-, gender- and BMI-matched control subjects, who have no diabetes, (age 34 ± 9 years; 20 men and 21 women) were also studied. Individuals with macrovascular complications (previous stroke or TIA, plaques on the carotid artery, heart disease and peripheral arterial disease), migraine, or proteinuria were excluded. The mean disease duration was 15 ± 10 years. Of the patients 19 had ≤ 10 years and 23 had > 10 years disease duration. All patients were treated with insulin, according to a routine clinical protocol. All diabetic patients have been treated at the Diabetes

Outpatient Service of the 2nd Department of Internal Medicine, Semmelweis University, Budapest.

Carotid Ultrasonography

Diameter and pulsatile distension of both CCA and the IMT of the posterior wall were measured with ultrasonography. The linear scanner was positioned 1 cm proximal to the bifurcation and a 1-cm segment was measured. IMT was examined between the leading edge of the first echogenic line (lumen-intima interface) and the second echogenic line (upper layer of the adventitia or media-adventitia interface) in the far artery wall at the best image quality. The transducer was in the mediolateral or lateral position. The ultrasound device (7.5 MHz linear array, Scanner 2000, PIE Medical, Maastricht, The Netherlands) was connected to a PC-based vessel wall echo-tracking system (Wall Track System, PIE Medical, Maastricht, The Netherlands). The built-in ECG signal was used to detect minimal wall displacement (D, end-diastolic diameter) between R wave signals.

In each 1-cm segment, 11 measurements were performed at 1-mm increments. At each point of measurement a 6-sec recording was performed containing 5-8 heart cycles (depending on heart rate). IMT values were obtained at the end of diastole. Therefore, for each patient, the mean of the 22 IMT (11 for each side) values x 5 to 8 distension pulses could be measured and averaged. In every patient, all carotid examinations were assessed by the same investigator, who was unaware of subjects' clinical details (diabetic patient or control).

Carotid Artery Pulse Pressure

CCA pressure was measured by applanation tonometry with a pencil-shaped probe (SPT-301, Millar Instruments, Houston, Texas) connected to the SphygmoCor system (AtCor Medical Pty Ltd, Sydney Australia). Carotid pulse wave recording was calibrated by using diastolic and mean BP values measured by sphygmomanometry on the right brachial artery. The carotid tonometric pressure was used to calculate carotid artery elastic parameters.

Pulse Wave Velocity

The carotid-femoral pulse wave analysis was carried out by applanation tonometry and simultaneous ECG recording. The SphygmoCor system (AtCor Medical Pty Ltd, Sydney Australia) calculated the time elapsed between the initial portion of the carotid/femoral pulse wave and the preceding ECG R-wave. The distances between the carotid/femoral measurement sites and jugular notch were defined with a standardized tape-measure. The averages of three PWV measurements were calculated.

Carotid Artery Elastic Variables

Carotid compliance coefficient (C) was calculated as $\Delta d/\Delta P$, where Δd is the change in diameter from end diastole to peak systole (distension), and ΔP is carotid pulse pressure. The distensibility coefficient (D) was calculated as $2\times\Delta d/(d\times\Delta P)$, where d is end-

diastolic diameter. Strain was calculated as the relative change in diameter during pulsatile distension as $100\times\Delta P / d$. Stiffness index β was expressed as $\ln(\text{SystolicP}/\text{DiastolicP})\times d/\Delta d$, where SP and DP are systolic and diastolic carotid pressure, respectively. The maximal end-diastolic carotid artery lumen cross-sectional area (LCSA) was calculated as $\pi(d/2)^2$. Intima-media cross-sectional area (IMCSA), which means the territory of the intima-media cross-sectional ring was calculated as $\pi(d/2+\text{IMT})^2 - \pi(d/2)^2$ at the end of diastole. This ring is situated outside of the lumen of CCA. The inner border of this ring is between lumen (blood) and intima and the external interface is between media and adventitia. Incremental elastic modulus was determined as $E_{inc}=[3[1+\text{LCSA}/\text{IMCSA}]]/D$ [19].

Duration of diabetes mellitus

Our patients were divided by the duration of diabetes by the 10-year cutoff used in the aforementioned studies, to compare different features of CCA across 3 groups (controls, diabetes ≤ 10 years and > 10 years disease duration). When predictors of morphological and functional features in T1DM patients were evaluated, disease duration was given in years from diagnosis.

Anthropometric parameters, blood sample, cardiovascular risk factors

Body height and weight were measured and BMI was calculated.

Fasting blood glucose, HbA_{1C}, total cholesterol, HDL-cholesterol, triglycerides, LDL-cholesterol, white blood cell count, C-reactive

protein, uric acid, cystatin C, B-type natriuretic peptide (BNP), thyroid-stimulating hormone (TSH) and micro- and macroalbuminuria were measured. Blood and urine samples were obtained within 7 days of the carotid examination.

Cardiovascular risk factors were evaluated by a questionnaire (hypertension, heart disease, smoking, hyperlipidemia, peripheral artery disease, migraine, stroke, alcohol consumption).

2. Methods of the study of patients with gestational diabetes mellitus

Subjects

Two groups of women in singleton pregnancy were examined in this cross-sectional study in the 3rd trimester at 34-36 weeks. Twenty-five women of GDM (n = 25, age 33 ± 4 years) and seventeen pregnant women of normal carbohydrate tolerance (n = 17, age 31 ± 5 years) were examined in this study. The women included in the study did not have a history of diabetes mellitus or a previous GDM. Control pregnant women were age- and BMI-matched. GDM was diagnosed between weeks 24 and 28 with a 75-g 2-hour oral glucose tolerance test (OGTT) using the WHO criteria in each pregnant women. All pregnant women were recruited from the 1st Department of Obstetrics and Gynecology and all diabetic women have been treated at the Diabetes Outpatient Service of the 2nd Department of Internal Medicine, Semmelweis University, Budapest.

Arterial wall properties of CCA

End-diastolic diameter (*d*), pulsatile distension (Δd) of the left common carotid artery was determined by ultrasonography. 1.0 cm, longitudinal segment of the CCA was insonated ~1 cm proximal from the bifurcation with a 7.5-10 MHz linear scanner (L10-5, 40mm, Picus Pro, Esaote, Netherlands). The radiofrequency (RF) signal of the ultrasound device was analyzed with a dedicated data acquisition hardware and software (Art.Lab, Esaote, The Netherlands). This novel system determines all arterial parameters (*d*, Δd, IMT) with very high precision and reproducibility in real time. IMT was examined between the leading edge of the first echogenic line (lumen-intima interface) and the second echogenic line (upper layer of the adventitia or media-adventitia interface) in the far artery wall at the best image quality. Analyzing the distension wave morphology, the software can detect the cardiac cycles even without ECG trigger. Based on the subject's heart rate, 4-7 distension waves are recorded in each session (6 s). With each subject, 3 sessions were recorded and cIMT and *d* results were averaged.

For the SI α determination "fast B-mode" scanning is utilized with the ultrasound scanner's (Picus Pro). These parameters were recorded during three, 6 seconds sessions and the results were averaged.

Carotid Artery Pulse Pressure, Carotid Artery Elastic Variables

Functional parameters were calculated with the same method as in the T1DM study. The Stiffness index α (SIα) was calculated as

ln(SPc/DPc)xA/ΔA. Area (A) is calculated automatically by Art.Lab software, this is the same parameter with the maximal end-diastolic carotid artery lumen cross-sectional area (LCSA) was calculated as $\pi(d/2)^2$.

Anthropometric parameters, Blood Sample, Cardiovascular Risk Factors

There were calculated with the same method as in the T1DM study.

3. Protocol

Subjects were studied two hours after eating in the early afternoon under standardized conditions, in a quiet room at a comfortable temperature. Upon arrival at the investigation unit the subjects filled out a questionnaire, then were equipped with measurement devices. Then they rested in the supine position for approximately 15 min until hemodynamic parameters returned to normal. The protocol began with the carotid measurements. Carotid artery tonometry was carried out after diameter and IMT determination on both sides. Then carotid-femoral PWV was determined.

Arterial examination was carried out in the 3rd trimester between weeks 34 and 36 in pregnant women.

4. Statistical analysis

For each subject the mean values of the right and left CCA were used. Data are expressed as mean ± SD. Risk factor distribution was compared by the Fisher-test. Normality of continuous variables was

checked by the Shapiro-Wilk test. Analysis of variance was used to evaluate data with normal distribution. The Tukey-test was applied for post hoc comparisons. For variables with non-normal distribution the Mann-Whitney test and the Spearman Rank Order correlation were used. General linear model (GLM) was applied to test for independent predictors of morphological and functional characteristics in the diabetic group. Control subjects were excluded from this analysis, and duration of diabetes was handled as a continuous variable. In the first step, Spearman rank order correlations were calculated. Those features that were significantly associated with *d*, IMT, IMCSA, SI and PWV, were entered in the multivariate analysis (GLM). Statistical significance was assumed at $p < 0.05$. Statistica for Windows v8.0 (StatSoft, Inc, Tulsa, OK) was used for data analysis.

RESULTS

1.Results of study of patients with type 1 diabetes mellitus

Participants of the control (41) and the diabetic (42) groups were successfully matched for gender, age and BMI. Systolic blood pressure (125 ± 14 vs. 132 ± 15 mmHg, $p = 0.031$), mean blood pressure (90 ± 10 vs. 94 ± 9 mmHg, $p = 0.040$) and heart rate (72 ± 12 vs. 81 ± 14 beats/min, $p = 0.002$) were significantly higher in diabetic patients.

All patients were treated with insulin, according to a routine clinical protocol. The circadian insulin demand (U/day) did not differ significantly in the shorter and longer duration groups (53 ± 19 vs. 55 ± 14 U, p = 0.71).

We divided our diabetic patients by the duration of diabetes by the 10-year cut-off. 19 patients were in T1DM ≤ 10 years and 23 were in T1DM > 10 years group. Groups were successfully matched for gender, age and BMI. The clinical characteristics did not differ between those with ≤ 10 years and with > 10 years disease duration.

Except for diabetes, the presences of cardiovascular risk factors were similar in control and diabetic patients.

Metabolic features

At the time of the study, we used intensive insulin therapy (insulin injected 4 times a day) in each diabetic patient. Fasting blood glucose and HbA_{1C} were significantly higher in diabetic patients and similar in the shorter and longer duration diabetic groups. White blood cell (WBC) counts were in the normal range, but significantly higher in the group of 42 T1 diabetic patients (6.68 ± 1.84 vs. 7.51 ± 1.84, p<0.043). There were no significant differences between groups in the other parameters.

Structural and functional characteristics of the common carotid artery in T1 diabetic patients

Significantly larger IMT (523±55 vs. 567±89 μm, p<0.01), IMCSA (11.60±1.81 vs. 13.08±3.02 mm^2, p<0.01), SI β (5.58±1.24 vs.

7.08±2.69, p<0.01) and PWV (6.00±0.82 vs. 6.61±1.56 m/s, p<0.05) were found in T1DM compared to controls. When T1DM patients with short and long disease duration (≤ or > 10 years) were compared with ANOVA, *d* (6450±433 vs. 6847±750 μm, p<0.05), IMCSA (11.97±1.98 vs. 14.01±3.43 mm, p<0.05) and PWV (5.90±0.92 vs. 7.20±1.74 m/s, p<0.01) differed significantly. After it the post hoc Tukey-test was used and only IMCSA remained statistically significantly. PWV also differed significantly with the post hoc Tukey-test, and the higher mean value suggested stiffening of large elastic arteries (aorta, carotid and femoral arteries) in patients who had long-standing diabetes. There was no significant difference in the other morphological and functional parameters among the 3 groups.

Predictors of morphological and functional features in T1DM

When multivariate analyses were restricted to T1DM patients, age was an independent predictor of SI and PWV, the duration of DM of IMCSA and PWV, systolic blood pressure of *d* and PWV, and LDL-C of IMT, IMCSA and SI.

2. Results of study of patients with gestational diabetes mellitus

Participants of the control and the GDM groups were successfully matched for age and BMI. Twelve women with GDM were taking 15 (1-78) Unit of insulin in different preparations and 200 g carbohydrate diet daily. The rest of 13 women with GDM were treated without insulin and 180-200 g carbohydrate diet was enough

to maintain normoglycaemia. HbA_{1C} levels indicated good glycemic control (5.36 ± 0.47 mmol/L).

Except for diabetes, the presence of cardiovascular risk factors was similar in women with GDM and control subjects. Three women had migraine and 1 woman had hypertension in the control group. Hypertension was treated with methyldopa. Four pregnant women had migraine and 1 woman had hyperlipidemia in the GDM group. Heart disease, smoking, peripheral artery disease and stroke did not occur in the study subjects. Alcohol consumption was not relevant in any group (0.1 vs. 0.2 U/day).

Gestational age was similar in the two groups (39 ± 1 weeks is both groups). Macrosomia, shoulder dystocia and birth trauma were not detected after birth. The number of instrumental delivery as Cesarean delivery did not differ significantly. There was not perinatal mortality and the Apgar scores were normal at 5 minutes. Preeclampsia was observed in one occasion in both groups.

Metabolic features

At the time of the OGTT the glucose level was normal at 0 minute (4.56 ± 0.6 vs. 5.04 ± 0.33 mmol/L, p=0.006) and of course it was significantly elevated at 120 minutes in the GDM group compare to control subjects (6.16 ± 0.84 vs. 9.62 ± 1.64 mmol/L, p<0.0001). Intensive insulin therapy and /or diet were introduced after the diagnosis. In both groups, namely in treated women with GDM and in unaffected pregnant women normal fasting blood glucose level (4.99 ± 0.51 vs. 4.79 ± 0.61 mmol/L, p=0.368) and HbA_{1C} proved

normoglycemia (5.33 ± 0.27 vs. 5.36 ± 0.47 mmol/L, p=0.846) in the 3rd trimester. There were no significant differences between groups in other parameters.

Structural and functional characteristics of the common carotid artery

Neither morphological (*d*, cIMT, IMCSA) nor functional (Δd, ΔP, strain, CC, DC, SI α and β, E_{inc}) characteristics of large elastic arteries differ significantly comparing women with GDM and unaffected controls in 3rd trimester. All parameters suggested some alteration of CCA in women with GDM but the differences were not significant for any feature.

CONCLUSION

1. Study of type 1 diabetes mellitus

1.1. In the present study, we investigated morphological and functional characteristics of large elastic arteries in type 1 diabetic patients without macrovascular complications. We found that of the morphological features IMT, diameter and IMCSA, whereas of the functional features stiffness and PWV are markers of macrovascular damage already in the presymptomatic stage.

1.2. When the diabetic group was divided into a shorter and a longer duration subgroup with a cutoff of 10 years disease duration, vessel diameter, IMCSA and PWV appeared to change after long lasting disease duration, whereas IMT and stiffness had a tendency to change already in the first decade after disease onset.

1.3. Our study points at the usefulness of the measurement of IMCSA, which is also related to *d* in addition to IMT.

1.4. There are differences in the time course of evolution and in predictors of morphological and functional changes in arteries in T1DM. LDL-cholesterol was an independent predictor of IMT, IMCSA and SI in our diabetic patients, i.e. LDL-C was an independent predictor of both morphological and functional features. The results of our study confirm the important role of LDL-cholesterol in atherogenesis.

2. Study of gestational diabetes mellitus

2.1. The strength of this study is the parallel evaluation of a range of morphological and functional features of CCA in women with GDM without clinically manifest macrovascular complications.

2.2. Neither morphological nor functional characteristics of large elastic arteries differ significantly between well-treated normoglycemic women with GDM and non-diabetic pregnant women in the 3rd trimester.

2.3. Similarly to previous reports that detected some alterations of large arteries in GDM our result also suggest that intensive treatment of carbohydrate intolerance and effective glycemic control in GDM may help to prevent early damage of large elastic arteries. On the other hand, we assumed that approximately 10-14 weeks period of GDM is not long enough time to the manifestation of changes of arteries.

PUBLICATIONS

Publications related to the thesis – Journal publications

1. Vastagh I, Horváth T, Nagy G, Varga T, Juhász E, Juhánsz V, Kollai M, Bereczki D, Somogyi A. (2010) Evolution and predictors of morphological and functional arterial changes in the course of type 1 diabetes mellitus. Diabetes Metab Res Rev 26: 646-655.

2. Vastagh I, Horváth T, Gamavölgyi Z, Rosta K, Folyovich A, Rigó J Jr, Kollai M, Bereczki D, Somogyi A. Preserved structural and functional characteristics of common carotid artery in properly treated normoglycemic women with gestational diabetes mellitus. Acta Physiol Hung No.13/11; 2011 (In press), DOI: 10.1556/APhysiol.98.2011.3.6

3. Vastagh I. (2009) A carotisstenosis ellátása LAM 9(3): 107-210.

4. Vastagh I. (2009) Az arteria carotis szűkületének jelentősége és kezelése. Studium & Practicum 6: 4-7.

5. Bereczki D, Vastagh I. (2011) A perifériás és a centrális vérnyomás klinikai jelentősége a neurológus szemszögéből. Hypertonia és Nephrologia 15(1): 11-15.

6. Vastagh I. (2011) Korai makrovaszkuláris szövődmények és azok vizsgálata cukorbetegségben. Metabolizmus 1:57-60.