

Cerebrovascular reactivity, IMT and nephropathy presence in patients with type 1 diabetes

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Submitted 2 October 2008 and accepted 6 February 2009.

This is an uncopyedited electronic version of an article accepted for publication in *Diabetes Care*. The American Diabetes Association, publisher of *Diabetes Care*, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of *Diabetes Care* in print and online at <http://care.diabetesjournals.org>.

Objective: Cerebrovascular reactivity impairment was reported as a marker of cerebral microangiopathy in long term type 1 diabetes. Intima-media complex thickening reflects early stages of macroangiopathy in type 1 diabetes. The analysis of the relationship between these variables and other microangiopathic complications might serve as a beneficial indicator for early prophylaxis in these patients.

Research Design and Methods: Vasomotor reactivity reserve (VMRr) and breath holding index (BHI) of the middle cerebral artery were measured with use of transcranial Doppler in 59 patients (median of age 32.0 years, range 20-51, 36 females) with type 1 diabetes, without history of cerebrovascular events and 30 healthy control subjects (median of age 31.5 years, range 25-39, 15 females). The relationships between the presence of selected vascular complications of type 1 diabetes and biochemical parameters, intima-media thickness (IMT) and VMRr and BHI in patients were analyzed.

Results: VMRr and BHI were lower in patients with type 1 diabetes when compared to healthy subjects (81.5% vs 100%, $p < 0.01$, and 1.6 vs 2.2, $p = 0.04$, respectively, whereas IMT was significantly higher in patients than in healthy controls (0.36 vs 0.30 mm, $p = 0.001$). However, no association of IMT with VMRr was found. We found a significant reduction of VMRr and BHI in patients with diabetic nephropathy.

Conclusions: The presence of diabetic nephropathy, but not IMT, can be regarded as an indicator of cerebral microangiopathy severity in patients with type 1 diabetes.

Stroke caused by small vessel disease can be observed more frequently in patients with diabetes mellitus than in general population (1). The presence of systemic microvascular complications of diabetes mellitus, especially nephropathy and retinopathy, also increases the risk of stroke and silent cerebral ischemia (2-3). Cerebral microangiopathy can be reflected by cerebrovascular reactivity (CVR) impairment (4). Previous studies on CVR in patients with type 1 diabetes have shown a reduced CVR in children with diabetic ketoacidosis and in adults with long duration of type 1 diabetes (5-6). A study using the intravenous 133 Xenon method showed CVR impairment in patients type 1 diabetes with nephropathy and retinopathy (7). In contrast, a single report revealed preserved CVR in asymptomatic adolescents with type 1 diabetes (8).

In spite of a high incidence of carotid artery disease and a high annual rate of intima-media thickness (IMT) progression in patients with type 1 diabetes, no information exists on the correlation between IMT and CVR to date (9-12). A single report published recently suggested the lack of such correlation in patients with type 2 diabetes (13). It is not clear if this result can be extrapolated to patients with type 1 diabetes.

Therefore, the aim of our study was to evaluate CVR in patients with type 1 diabetes without advanced diabetic complications, and to assess the relationship between the CVR and type 1 diabetes course and the presence of diabetic macro- and microangiopathy.

RESEARCH DESIGN AND METHODS

Patients and controls: The study population consisted of 59 patients with type 1 diabetes (36 women and 23 men, median age 32.0 years, range limits 20-51 years), recruited from the Regional Diabetological Centre of our university and 30 healthy

volunteers (15 women and 15 men, median age 31.5 years, range limits 20-39 years). We included patients with minimal diabetes duration of 2 years, free of focal neurological deficits, respiratory tract disease (at present and in history) and renal insufficiency. We excluded patients with past cerebrovascular events or head trauma, pregnant and those with incidents of hypoglycemia within 30 days prior to the study. Current and past smokers were also excluded in both patients and controls groups, as well as heavy drinkers and users of any hormonal therapy within 30 days prior to study.

All examinations were performed at the same time of the day (between 10.00 AM and 14.00 PM). All study subjects were asked to stop drinking coffee upon examination day, and to avoid sleep deprivation and fasting.

The study protocol included history taking, neurological examination, extracranial and transcranial ultrasound, fundoscopy and laboratory testing. The study protocol was approved by the Medical Ethics Committee of The Medical University of Gdansk (NKEBN/3/2005). Upon entry, each participant gave her/his informed consent.

Subjects characteristics: Patient's history was obtained including the information on past and current disorders as well as on co-morbid conditions. The weight and height were recorded and expressed as the body mass index (BMI). Focal neurological deficits were excluded by neurological examination performed by neurologists certified by the Polish Neurological Society. Diabetic neuropathy was diagnosed with the criteria of Neuropathy Symptoms Score (NSS), based on patients' complaints and neuropathic deficits found on neurological examination (14). Hypertension was diagnosed if two consecutive measurements of systolic and diastolic blood pressures exceeded 130 mmHg and 80 mmHg respectively or if anti-hypertensive

medication was used. Laboratory examinations in patients with type 1 diabetes included measurements of circadian urinary protein/microalbuminuria, total serum cholesterol and glycated hemoglobin (HbA1c). The biochemical examinations were performed 1 month (+/-7 days) prior to TCD. Hyperlipidaemia was diagnosed, if total cholesterol and/or triglycerides exceeded 175 mg/dL and 150 mg/dL respectively; or cholesterol/triglycerides-lowering medications were used.

Based on American Diabetic Association (ADA) criteria microalbuminuria was defined as excretion of 30–300 mg of albumin/24 h on two of three urine collections repeated at intervals of up to 6 months (15). Clinical albuminuria or “overt nephropathy” per the ADA recommendations corresponded to protein excretion over 300 mg/24 h. In our patients, quantitative measurements of urine protein excretion in a sample obtained from 24 hours urine collection were used to assess the severity of proteinuria. Albumin concentration in urine were measured with turbidimetric method. We included in the statistical analysis the measurement, which was closest in time to the TCD study.

Fundoscopy: Retinopathy was recognized on fundoscopy performed by an ophthalmologist certified by Polish Ophthalmic Society. Grading was made with the use of The Stages of Diabetic Retinopathy of American Academy of Ophthalmology (AAO). Previous therapy with photocoagulation was also recognized as a marker of diabetic retinopathy (16).

Intima-media Thickness Measurement

The IMT in both carotid arteries was measured using a Aloka 5000 ultrasound machine (ALOKA.Co., Ltd, Japan) equipped with a linear probe with central working frequency of 7.5 MHz and range limits of 5–10 MHz, after standard examination of both carotid arteries. During each examination, the distal 2 cm-long segment of the common

carotid artery in 2 different (anterior-posterior and lateral) longitudinal projections was assessed bilaterally. The final value of IMT was calculated as a mean of 4 measurements (2 projections both sides). IMT was measured off-line using a semi-automatic method (Carotid Measure System) performed by one investigator (KBK, 12 years of experience) who was blinded to the patients’ history.

Transcranial Doppler examination: The middle cerebral artery (MCA) flow parameters were measured using MultiDop T2 ultrasound machine (DWL Elektronische Systeme GmbH, Singen, Germany), equipped with the 2 MHz pulse wave probe. Velocity measurements were performed simultaneously in both MCA with the use of a two-channel monitoring kit: 2 probes 2 MHz PW, the fixation band and the monitoring program (MF version 8.27 L, DWL). The physiological techniques of provoking cerebrovascular reactivity by changes of pCO₂ were applied according to the published standards (17-18). During the CO₂ reactivity test, the CO₂ content in expired air (end tidal CO₂ concentration) was monitored continuously (capnograph Datex Normocap, Finland). Before and after the tests, the systemic blood pressure and heart rate were measured. The vasomotor reactivity reserve (VMRr) expressed in % of change from baseline and breath holding index (BHI) were calculating according to the standard protocol published previously (17-18). The median values of arithmetical means of velocity measurements at rest (rest Vmean), VMRr, BHI, Pulsatility Index (PI) and Resistance Index (RI) of both MCAs were used for further analyses.

Reproducibility: In 9 healthy volunteers, the reproducibility of VMRr measurement was checked. The unbiased intra-class correlation coefficient (ICC) for the results of two consecutive measurements of VMRr was considered acceptable (ICC= 0.89, p=0.007).

Statistics: All the analyses were performed with the STATISTICA, version 7.1 StatSoft Inc, 2005 (SN ABDP509753932AR). Shapiro-Wilk tests were performed to analyze the distribution of continuous variables. Differences between groups were analyzed with Student's t-test in case of normally distributed variables (type 1 diabetes duration) or with Mann-Whitney U test in case of not-normally distributed variables (age, BMI, diastolic and systolic blood pressure, heart rate, VMRr, BHI, PI, RI, end tidal CO₂ concentration and IMT). The chi-square test was used to compare a proportion of females to males in groups. Correlation was assessed by Spearman rank correlation test. Multivariable linear regression analysis was used to assess confounders of CVR in patients with type 1 diabetes. The level of $p < 0.05$ was regarded as statistically significant.

RESULTS

All patients and volunteers were free of coronary heart disease, diabetic foot, orthostatic hypotension, chronic renal insufficiency or hemodynamically significant stenosis (>50%) of extra- and intracranial cerebral arteries as determined by physical check, biochemical analysis, carotid and transcranial ultrasound, respectively. There was no significant difference regarding age, gender, BMI, blood pressure and heart rate between patients and healthy controls (Table 1).

The median values of VMRr and BHI were significantly lower in patients than in the healthy controls. Both groups did not differ regarding values of rest Vmean, PI and RI. The end tidal CO₂ concentrations did not differ between the groups, both during hyperventilation and breath-holding. The median value of IMT in patients was significantly higher in comparison to respective values in healthy controls (Table 2). We found no significant correlations between VMRr or BHI values and age,

diabetes duration, daily insulin demand, HbA1c concentrations, total serum cholesterol, triglycerides level or IMT in patients with type 1 diabetes. Furthermore, no significant correlations between IMT and VMRr or BHI were seen in healthy controls.

Taking together both microalbuminuria and overt nephropathy into one group, an analysis showed a significant reduction of CVR values in the presence of diabetic nephropathy (Table 3). After exclusion of two patients with overt nephropathy, VMRr remained impaired in patients with microalbuminuria when compared to those without microalbuminuria (71.0 vs 87.5 %, $p=0.03$).

The median age patients with nephropathy was 30 years, min 27, max 45 years, while in patients without nephropathy 32 years, min 20, max 51 years. The age difference between the two groups was not significant ($p=0.69$). Mean duration of type 1 diabetes in patients with nephropathy (18.3 ± 7.8) was not different from the duration in patients without nephropathy (15.1 ± 6.4 years, $p=0.24$).

We found no significant differences of CVR values between the subgroups of patients with type 1 diabetes distinguished on the basis of the presence of diabetic retinopathy, neuropathy, hypertension and hyperlipidaemia. Additionally, no differences regarding VMRr or BHI were found between groups of patients distinguished on the basis of use of statins or angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (Table 3).

The multivariable regression analysis confirmed a significant, inverse association between diabetic microalbuminuria/nephropathy and VMRr independently from other vascular risk factors or type 1 diabetes related organ damage (Table 4).

CONCLUSIONS

Our study shows a reduction of VMRr and BHI in patients with type 1 diabetes, who were free of overt cerebrovascular events. These CVR parameters were significantly lower in the subgroup of patients with diabetic nephropathy. However, CVR was not associated with diabetes course, presence of additional risk factors, biochemical parameters, and intima-media thickening.

We revealed that the presence of diabetic nephropathy can indicate sub-clinical cerebrovascular pathology in patients with type 1 diabetes independently from other micro- and macroangiopathy evidences. Our findings are in line with results of a previous study, which demonstrated a close relationship between indices of cerebral microangiopathy (PI and CVR) and microalbuminuria in patients with type 2 diabetes mellitus (19). Few studies have reported an association between CVR and diabetic retinopathy in both type 1 diabetes and type-2-diabetes (7, 13). We also found a similar trend with borderline significance towards a CVR impairment in presence of diabetic retinopathy. However, lack of statistical significance can probably be explained by type 2 error due to the small sample size. Interestingly, the relationship between CVR and nephropathy in our patients was not influenced by hypertension, a finding which has also been described in former studies on type 1 and type 2 diabetes (6, 13). Furthermore, we did not find any relationship between use of antihypertensive or statin treatment and CVR our patients, though statin and angiotensin-converting enzyme inhibitor treatments were reported as potential CVR confounders (20-21). The lack of confounders effects can be explain by relatively small sample size in our study.

The lack of correlation between IMT and CVR seen in our study has also been observed in patients with type 2 diabetes (13). To our best knowledge no published data exists on this correlation in patients with type

1 diabetes. However, recently published research showed negative correlation of cerebrovascular reactivity to L-arginine with IMT. This approach may be an alternative method for analysis of CVR and IMT relationship in patients with type 1 diabetes (22).

The provocation methods used in our study to estimate the reactivity of cerebral vessels (hyperventilation and breath holding tests) have not been published yet for the adult patients with type 1 diabetes. Advantages of this approach are the simulation of the natural cerebrovascular autoregulation mechanisms resulting from the use of a physiological method of evoking changes in pCO₂, safety, noninvasiveness and lack of side effects. The VMRr enabled us to assess a full range of vasodilatation, instead of assessing only the upper limit obtained during the hypercapnia, as it was previously published in patients with type 1 diabetes (5-6, 8). The use of these tests allowed us to detect CVR impairment in the whole group of patients, and not only in subgroups with longer duration of type 1 diabetes or with higher complications rate, as it was previously presented (5-7). Also, to avoid a possible influence of smoking and hormonal replacement therapy on CVR we limited our study to non-smokers and non hormonal users (23-24). In previous studies, the confounding effects of smoking and hormonal therapy were not controlled for (6, 13).

As a limitation of the presented study we should mention that a relatively small sample size allowed us to detect only a single factor that has a significant impact on CVR in the examined group patients. The correlation between the presence of diabetic retinopathy and the low CVR values found in some studies could not be confirmed by our data (6, 7). However, both the univariate and multivariate analyses have shown statistical significant correlation between diabetic nephropathy and CVR impairment. This trend

is also in agreement with previously published data (7, 19). Despite a relatively large proportion of hyperlipidaemic patients in our study, no influence of hyperlipidaemia on CVR was found, similarly to previous reports (6, 25).

The presence of clinically asymptomatic CVR impairment in a group of patients with type 1 diabetes and peripheral microcirculatory dysfunction is an argument for early implementation of primary stroke prevention in these patients.

ACKNOWLEDGEMENTS

For realization of the study the financial resources of Medical University of Gdansk were used (grant KBN nr W-753).

The authors gratefully acknowledge Prof. James H. Halsey (Department of Neurology, The University of Alabama and Birmingham) for helpful advise and suggestions. The authors also acknowledge Dr. Janja Pretnar-Oblak (Department of Neurology, Ljubljana Medical Centre, Ljubljana, Slovenia) for helpful suggestions.

Disclosure: Authors have no relevant conflict of interest to disclosure.

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Table 1. Characteristics of patients with type 1 diabetes and healthy controls.

	patients	healthy controls	P value
n	59	30	
Age [years]	32.0 (20-51)	31.5 (25-39)	0.37
F:M ratio	36:23	15:15	0.32; Chi ² NW=0.98
BMI [kg/m ²]	23.9 (20-31.6)	23.2 (19.2-33)	0.18
Systolic blood pressure [mm Hg]	120 (98-145)	120 (100-140).	0.68
Diastolic blood pressure [mm Hg]	80 (68-98)	77 (60-94)	0.11
Heart rate [beats/min]	73 (55-98)	69 (50-88)	0.3
Hyperlipidaemia [%]	61.0		
Hypertension [%]	16.0		
Neuropathy [%]	20.3		
Retinopathy[%]	44.1		
Range of albuminuria [mg/day]	0.45-2029		
Microalbuminuria[%]	13.6		
Overt nephropathy [%]	3.3		
Type 1 diabetes duration [years]	15.0 (3-33)		
HbA1C [%]	7.8 (5.9-12)		
Circadian insulin demand [units]	49 (19-82)		
ACEI or ARB treatment [%]	15.2		
Statin treatment [%]	6.7		

F:M ratio – females to males ratio; BMI–body mass index, HbA1c- glycated hemoglobin; ACEI - angiotensin-converting enzyme inhibitor; ABR - angiotensin receptor blocker

Table 2. The comparison of the MCA blood flow parameters and IMT between patients with type 1 diabetes and healthy controls.

	patients	healthy controls	P
n	59	30	
rest Vmean [cm/sec]	60.7 (35.7–106.5)	57.3 (36.8–85.9)	0.27
Pulsatility index	0.88 (0.65–1.52)	0.89 (0.67–1.20)	0.19
Resistance index	0.81 (0.44–0.75)	0.57 (0.49–0.69)	0.36
VMRr [%]	82.9 (39–143)	100 (52-142)	0.006
BHI	1.62 (0.42–3.41)	2.18 (0.89-3.63)	0.045
Delta etCO ₂ HV[%]	1.65 (1.0-2.5)	1.75 (1.0-3.0)	0.23
Delta etCO ₂ BH [%]	1.1 (0.5-2.0)	1.1 (0.7-1.8)	0.94
IMT [mm]	0.35 (0.26–0.64)	0.30 (0.21–0.43)	0.001

rest Vmean – mean flow velocity measured at rest; VMRr – vasomotor reactivity reserve; BHI–breath holding index; BMI – body mass index; Delta etCO₂HV – delta end tidal CO₂ during hyperventilation; Delta etCO₂ BH - delta end tidal CO₂ during breath holding; IMT – intima-media thickness

Table 3. The impact of vascular risk factors and co-morbidities on CVR parameters in patients with type 1 diabetes.

Co-morbid condition	VMRr [%]			BHI		
	present	absent	P	present	absent	p
Hyperlipidaemia, n=36	80.8	88.8	0.5	1.62	1.65	0.2
Hypertension, n=10	80.6	83.0	0.90	1.65	1.67	0.88
Neuropathy, n=12	82.5	81.5	0.49	1.58	1.65	0.34
Retinopathy, n = 26	82.3	92.3	0.09	1.47	1.72	0.08
Nephropathy, n = 10	73.9	83.5	0.029	1.37	1.69	0.036
ACEI or ARB treatment, n=9	77.6	83.2	0.32	1.61	1.65	0.56
Statin treatment, n=4	77.1	82.7	0.31	1.50	1.62	0.36

VMRr – vasomotor reactivity reserve; BHI–breath holding index; ACEI - angiotensin-converting enzyme inhibitor; ABR - angiotensin receptor blocker

Table 4. Results of multivariable linear regression analyses performed in group of patients with type 1 diabetes to assess VMRr confounders.

Variable, n	BETA	P
Gender	-0.08	0.59
Retinopathy, n = 26	-0.12	0.43
Nephropathy, n = 10	-0.33	0.044
Neuropathy, n = 12	0.30	0.053
Hypertension, n = 10	0.02	0.88
Hyperlipidaemia, n = 36	-0.01	0.93
HbA1c [%]	0.13	0.32

HbA1c- glycated hemoglobin