Cerebrovascular Reactivity, Intima-Media Thickness, and Nephropathy Presence in Patients With Type 1 Diabetes

Grzegorz M. Kozera, md, phd[1]
Bogumił Wolnik, md, phd[2]
Katarzyna B. Kunicka, md, phd[3]
Sebastian Szczyrba, md[1]

Joanna Wojczal, md, phd[4]
Ulf Schminke, md[5]
Walenty M. Nyka, md, phd[1]
Leszek Bieniaszewski, md, phd[3]

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 transcranial Doppler in 59 patients (median age 32.0 years, range 20–51, 36 females) with type 1 diabetes, without history of cerebrovascular events, and 30 healthy control subjects (median 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 with 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 then in healthy control subjects (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.

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Stroke caused by small-vessel disease can be observed more frequently in patients with diabetes than in the general population (1). The presence of systemic microvascular complications of diabetes, 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 ke-toacidosis 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 with 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 be-tween 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 diabetes 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 — 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), re-cruited from the Regional Diabetological Centre of our university and 30 healthy volunteers (15 women and 15 men, me-dian age 31.5 years, range limits 20–39 years). We included patients with mini-mal diabetes duration of 2 years and who were free of focal neurological deficits, re-spiratory tract disease (at present and in history), and renal insufficiency. We ex-cluded patients with past cerebrovascular events or head trauma, those who were pregnant, and those with incidents of hy-poglycemia within 30 days before the study. Current and past smokers were also excluded in both patients and control groups, as well as heavy drinkers and us-ers of any hormonal therapy within 30 days before study.

All examinations were performed at the same time of the day (between 10:00 A.M. and 2:00 P.M.). All study subjects were asked to stop drinking coffee on ex-amination day and to avoid sleep depri-vation and fasting.

The study protocol included history taking, neurological examination, ex-tracranial and transcranial ultrasound, fundoscopy, and laboratory testing. The study protocol was approved by the Med-ical Ethics Committee of the Medical Uni-versity of Gdańsk (NKEBN/3/2005). Upon entry, each participant gave in-formed consent.

Subject characteristics

Patient history was obtained, including the information on past and current disorders as well as on comorbid conditions. The weight and height were recorded and expressed as the 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, 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 and 80 mmHg, respectively, or if antihypertensive medication was used. Laboratory examinations in patients with type 1 diabetes included measurements of circadian urinary protein/microalbuminuria, total serum cholesterol, and A1C. The biochemical examinations were performed 1 month ($\pm$7 days) before transcranial Doppler examination (TCD). Hyperlipidemia was diagnosed if total cholesterol and/or triglycerides exceeded 175 and 150 mg/dl, respectively, or if cholesterol/triglyceride-lowering medications were used.

Based on American Diabetes Association criteria, microalbuminuria was defined as excretion of 30–300 mg 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 American Diabetes Association 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-h urine collection were used to assess the severity of proteinuria. Albumin concentrations in urine were measured with the turbidimetric method. We included in the statistical analysis the measurement that was closest in time to the TCD study.

Fundoscopy

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

Table 1—*Characteristics of patients with type 1 diabetes and healthy control subjects*

	Patients	Healthy control subjects	P
n	59	30	—
Age (years)	32.0 (20–51)	31.5 (25–39)	0.37
F:M ratio	36:23	15:15	0.32; χ^2 M-L = 0.98
BMI (kg/m^2)	23.9 (20–31.6)	23.2 (19.2–33)	0.18
Systolic blood pressure (mmHg)	120 (98–145)	120 (100–140)	0.68
Diastolic blood pressure (mmHg)	80 (68–98)	77 (60–94)	0.11
Heart rate (beats/min)	73 (55–98)	69 (50–88)	0.3
Hyperlipidemia (%)	61.0		
Hypertension (%)	16.0		
Neuropathy (%)	20.3		
Retinopathy (%)	44.1		
Range of albuminuria (mg/day)	0.45–2,029		
Microalbuminuria (%)	13.6		
Overt nephropathy (%)	3.3		
Type 1 diabetes duration (years)	15.0 ± 3–33		
A1C (%)	7.8 (5.9–12)		
Circadian insulin demand (units)	49 (19–82)		
ACE inhibition or angiotensin receptor blocker treatment (%)	15.2		
Statin treatment (%)	6.7		

Data are median (range), arithmetic mean $\pm$ SD, or percentage of patients. M-L, maximum likelihood.

IMT measurement

The IMT in both carotid arteries was measured using an Aloka 5000 ultrasound machine (ALOKA, 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 two different (anterior-posterior and lateral) longitudinal projections was assessed bilaterally. The final value of IMT was calculated as a mean of four measurements (two projections, both sides). IMT was measured offline using a semiautomatic method (Carotid Measure System) performed by one investigator (K.B.K., 12 years of experience) who was blinded to the patients' history.

Transcranial Doppler examination

The middle cerebral artery flow parameters were measured using the MultiDop T2 ultrasound machine (DWL Elektronische Systeme, Singen, Germany), equipped with the 2-MHz pulse wave probe. Velocity measurements were performed simultaneously in both middle cerebral arteries with the use of a two-channel monitoring kit: two probes 2-MHz pulse wave, the fixation band, and the monitoring program (MF version 8.27 l; DWL Elektronische Systeme). The

physiological techniques of provoking cerebrovascular reactivity by changes of pCO_2 were applied according to the published standards (17,18). During the CO_2 reactivity test, the CO_2 content in expired air (end tidal CO_2 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 percent 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 V_{mean}), VMRr, BHI, pulsatility index (PI), and resistance index (RI) of both middle cerebral arteries were used for further analyses.

Reproducibility

In nine healthy volunteers, the reproducibility of VMRr measurement was checked. The unbiased intraclass correlation coefficient for the results of two consecutive measurements of VMRr was considered acceptable (intraclass correlation coefficient = 0.89, P = 0.007).

Statistical analysis

All the analyses were performed with STATISTICA, version 7.1 (StatSoft, 2005;

Table 2—Comparison of the middle cerebral artery blood flow parameters and IMT between patients with type 1 diabetes and healthy control subjects

	Patients	Healthy control subjects	P
n	59	30	
Resting V_{mean} (cm/s)	60.7 (35.7–106.5)	57.3 (36.8–85.9)	0.27
PI	0.88 (0.65–1.52)	0.89 (0.67–1.20)	0.19
RI	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

Data are median (range). Δ etCO$_2$HV, Δ end tidal CO$_2$ during hyperventilation; Δ etCO$_2$ BH, Δ end tidal CO$_2$ during breath holding.

SN ABDP509753932AR). Shapiro-Wilk tests were performed to analyze the distribution of continuous variables. Differences between groups were analyzed with the Student's t test in case of normally distributed variables (type 1 diabetes duration) or with Mann-Whitney U test in case of non–normally distributed variables (age, BMI, diastolic and systolic blood pressure, heart rate, VMRr, BHI, PI, RI, end tidal CO$_2$ concentration, and IMT). The χ^2 test was used to compare a proportion of females to males in groups. Correlation was assessed by the 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, and carotid and transcranial ultrasound, respectively. There was no significant difference regarding age, sex, BMI, blood pressure, and heart rate between patients and healthy control subjects (Table 1).

The median values of VMRr and BHI were significantly lower in patients than in the healthy control subjects. Both groups did not differ regarding values of resting V_{mean}, PI, and RI. The end tidal CO$_2$ 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 control subjects (Table 2). We found no significant correlations between VMRr or BHI values and age, diabetes duration, daily insulin demand, A1C concentrations, total serum cholesterol, triglyceride level, or IMT in patients with type 1 diabetes. Furthermore, no significant correlations between IMT and VMRr or BHI were seen in healthy control subjects.

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 with individuals without microalbuminuria (71.0 vs. 87.5%, $P = 0.03$).

The median age in patients with nephropathy was 30 years (minimum 27 years, maximum 45 years), while in patients without nephropathy, it was 32 years (minimum 20, maximum 51). 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 years) 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 hyperlipidemia. Additionally, no differences regarding VMRr or BHI were found between groups of patients distinguished on the basis of use of statins or ACE 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 subclinical cerebrovascular pathology in patients with type 1 diabetes independently from other micro- and macroangiopathy evidence. Our findings are in line with results of a previous study, which demonstrated a close relationship between indexes of cerebral microangiopathy (PI and CVR) and microalbuminuria in patients with type 2 diabetes (19). Few studies have reported an association between CVR and diabetic retinopathy in both type 1 and type 2 diabetes (7,13). We also

Table 3—Impact of vascular risk factors and comorbidities on CVR parameters in patients with type 1 diabetes

		VMRr (%)			BHI		
	n	Present	Absent	P	Present	Absent	P
Hyperlipidemia	36	80.8	88.8	0.5	1.62	1.65	0.2
Hypertension	10	80.6	83.0	0.90	1.65	1.67	0.88
Neuropathy	12	82.5	81.5	0.49	1.58	1.65	0.34
Retinopathy	26	82.3	92.3	0.09	1.47	1.72	0.08
Nephropathy	10	73.9	83.5	0.029	1.37	1.69	0.036
ACE inhibitor or angiotensin receptor blocker treatment	9	77.6	83.2	0.32	1.61	1.65	0.56
Statin treatment	4	77.1	82.7	0.31	1.50	1.62	0.36

Data are median values.

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

	n	β	P
Sex		−0.08	0.59
Retinopathy	26	−0.12	0.43
Nephropathy	10	−0.33	0.044
Neuropathy	12	0.30	0.053
Hypertension	10	0.02	0.88
Hyperlipidemia	36	−0.01	0.93
A1C (%)		0.13	0.32

Serum concentrations in percent of A1C were taken.

found a similar trend with borderline significance toward a CVR impairment in the presence of diabetic retinopathy. However, lack of statistical significance can probably be explained by type 2 error because of the small sample size. Interestingly, the relationship between CVR and nephropathy in our patients was not influenced by hypertension, a finding that has also been described in former studies on type 1 and type 2 diabetes (6,13). Furthermore, we did not find any relationship between the use of antihypertensive or statin treatment and CVR in our patients, although statin and ACE inhibitor treatments were reported as potential CVR confounders (20,21). The lack of confounder effects can be explained by the 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 exist 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 pCO$_2$, safety, noninvasiveness, and lack of side effects. The VMRr enabled us to assess a full range of vasodilation, 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 nonsmokers and nonhormonal users (23,24). In previous studies, the confounding effects of smoking and hormonal therapy were not controlled (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 statistically 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 hyperlipidemic patients in our study, no influence of hyperlipidemia on CVR was found, similar 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.

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