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Impaired Cerebral Vasoreactivity in Type 2 Diabetes Mellitus

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A B S T R A C T

The aim of our study was to assess cerebral vasoreactivity (CVR) in type 2 diabetes mellitus (DM2) and factors which may influence on it. According to previous studies, evaluating CVR in DM2 on the similar way, the results were dubious. For the evaluation CVR we used breath holding index (BHI) and transcranial Doppler ultrasound (TCD) in 50 patients with DM2 and 50 sex- and age-matched healthy controls. We observed epidemiologic and clinic data, other vascular risk factors and laboratory parameters. We found statistically significant difference in BHI between patients with DM2 (BHI=0.69±0.31) and age- and sex- matched healthy controls (BHI=1.33±0.28) ($p<0.05$). Because of a significant correlation between BHI and age ($p<0.001$) in healthy controls we made an adjustment of BHI for age before further analyses (BHI_{adj}). In DM2 group we found a significant correlation between BHI_{adj} and age ($p=0.0004$), fasting glycemia ($p=0.04$), and albuminuria ($p=0.04$) (creatinine clearance in multivariate analysis ($p=0.007$)). Our study has shown that CVR is impaired in DM2 patients and that it's severity was associated with age, fasting glycemia and renal function. Functional TCD is a very good screening method for detection and monitoring of cerebral microangiopathic changes in DM2 patients.

Key words: *type II diabetes mellitus, Breath-holding index (BHI), cerebral vasoreactivity*

Introduction

Cerebrovascular diseases are the second cause of death and the leading cause of disability[1]. We are still dissatisfied with the therapy of stroke. Therefore, an early identification of cerebrovascular risk factors is needed. We are particularly interested in the diabetic patients, where relatively good treatment possibilities exist. As we know from previous studies, the risk of stroke is increased 2-to-3 fold in patients with the type 2 diabetes mellitus (DM2)[2] and if it is repeated, their stroke outcomes and prognosis are poor[3]. Scientific interest is more focused on changes in the big blood vessels in diabetic patients, but there are also changes in small ones. Cerebral autoregulation is the ability to maintain a constant cerebral blood flow (CBF) despite changes in the cerebral perfusion pressure and could be evaluated by the cerebral vasoreactivity (CVR), a hemodynamic parameter representing the increase in the CBF velocity in response to a vasodilatory stimulus, like hypercapnia[4,5]. Impaired cerebrovascular reactivity is an indicator of the increased risk of stroke[6–8]. Transcranial Doppler ultrasound (TCD) is good, noninvasive and accurate method for the evaluation of increase in mean flow velocity (MFV) in the cerebral arteries as a response to hypercapnia, induced by the breath-holding test (BHT)[9–16]. It is well known that patients with type 2 diabetes mellitus have impaired ability of the endothelium to properly maintain vascular homeostasis[17]. Furthermore, diabetic patients have increased risk of stroke as one of macrovascular complications of diabetes[2,3]. In previous studies, evaluation of the CVR in the DM2 in a similar way, has given controversial results. Some of them have shown no differences in the CVR between DM2 patients and healthy controls[18,19],

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and some of them have shown impaired the CVR but only in a group with the microangiopathic complications[20,21] and in a group of patients who had DM2 longer than 10 years[22]. The aim of this study was to assess cerebral autoregulation in type 2 diabetes mellitus (DM2) and the factors which may influence on it.

Materials and Methods

The study included 50 DM2 patients from outpatient clinic of Department of Endocrinology, Diabetes and Metabolic Disorders of Dubrava University Hospital and 50 sex- and age-matched healthy controls. Inclusion criteria were type 2 diabetes mellitus, age of 18 to 70, ability to participate in study and written Informed consent. The exclusion criteria were poor insonation of the temporal bone window, moderate or significant stenosis of the main neck blood vessels, excluded with extracranial Doppler ultrasound (Aloka 5500 Prosound), and non-cooperative, unconscious or demented patient.

The TCD examination was performed through transtemporal window at a depth of 50 mm, on a TCD DWL Multidop X4 (DWL Elektronische Systeme GmbH, Sipplingen, Germany) instrument with 2 MHz hand-held pulsed wave probe, in supine position after 5-minute bed rest. Each middle cerebral artery (MCA) was insonated separately by standard protocol[4,9–12] and mean flow velocity (MFV) values were recorded in the rest and during holding a breath for 30 seconds at the end of a normal inspiration. If they were not able to hold their breath for 30 seconds, they held it as long as they could and that time was taken in a subsequent calculation. Vascular reactivity to hypercapnia was studied by calculating the breath-holding index (BHI). The index is obtained by dividing the percent increase in the mean flow velocity (MFV) occurred during the breath-holding by the length of time (seconds) that subjects hold their breath after a normal inspiration ((MFV at the end of BHT – rest MFV/rest MFV)X100/second of breath-holding). Heart rate, arterial pressure in arm, SaO2 were measured as in previous studies are described[12,13]. Concerning the factors that may influence on the CVR, we observed: age, sex, other major vascular risk factors (hypertension, hyperlipemia, atrial fibrillation, mild stenosis (<50%) of the main neck blood vessels, obesity, smoking, consuming alcohol), previous neurological disturbances, diabetic complications (retinopathy, nephropathy and polyneuropathy), duration of diabetes, waist circumference and laboratory parameters (fasting glycemia and two hours after meal glycemia, glycated hemoglobin (HbA1c) total, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, C-reactive protein, hematocrit, hepatic enzymes (AST – aspartate aminotransferase; ALT – alanine transaminase; GGT – gamma-glutamyl transpeptidase), creatinine clearance and albuminuria in 24 hours urine). Statistical analyses were performed using STATISTICA ver. 6.0 (StatSoft, Inc. Tulsa, OK)[23] and MedCalc (MedCalc Software, Mariakerke, Belgium). Data were expressed as means and SD if normally distributed and as median and range if not. Normality of distribution was tested using Kolmogorov-Smirnov test. Prior to further analysis variables that didn't have normal distribution were normalized using logarithmic transformation. Between groups comparisons were made using Student's t-test. The linear regression analysis was perfor-

TABLE 1
THE CLINICAL AND LABORATORY CHARACTERISTICS OF DM2 PATIENTS

Variable	Mean (95% CI)	SD	Median	Minimum	Maximum
Age (years)	58.3 (54.9–61.9)	12.0	58.0	20.0	78.0
Duration (years)	9.6 (7.5–11.7)	7.4	9.5	1.0	33.0
Total cholesterol (mmol/L)	4.9 (4.6–5.3)	1.2	4.9	2.2	8.8
Triglycerides (mmol/L)	1.8 (1.5–2.1)	0.9	1.6	0.5	6.1
HDL cholesterol (mmol/L)	1.2 (1.1–1.3)	0.3	1.2	0.6	2.1
LDL cholesterol (mmol/L)	2.8 (2.6–3.1)	1.0	2.8	0.7	5.8
HbA1c (%)	9.0 (8.3–9.6)	2.1	8.5	5.5	13.7
Albuminuria (mg/day)*	191.1 (20.5–361.7)	600.1	10.9	0.1	3070.0
rsid7351224 Fasting glycemia (mmol/L)	7.6 (7.0–8.3)	2.2	7.2	4.1	14.2
2 hours after meal glycemia (mmol/L)	10.5 (9.6–11.4)	3.1	10.2	5.5	17.4
C-reactive protein (mg/L)*	5.3 (3.4–7.1)	6.5	2.2	0.2	25.6
Creatinine clearance (mL/s)	1.7 (1.6–1.9)	0.6	1.8	0.5	3.2
AST (U/L)	22.0 (19.5–24.5)	8.7	19.5	10.0	47.0
ALT (U/L)	28.5 (24.9–33.0)	15.8	24.5	6.0	87.0
Waist circumference (cm)	101.5 (97.3–105.7)	14.8	99.5	80.0	152.0
GGT (U/L)	46.3 (33.9–58.7)	43.5	31.5	10.0	173.0
Hematocrit L/L	0.406 (0.398–0.415)	0.03	0.408	0.324	0.460

All data are presented as mean (SD) or * median (range)

med to examine the relationship between BHI and age in healthy controls. Results of the linear regression analysis were used to adjust the BHI (BHI_{adj}) values in the DM2 group according to age before further analyses. Analysis of variance was used to assess the effects of other major vascular risk factors, previous neurological disturbances, and diabetic complications on the BHI_{adj} in the DM2 patients. Associated risk for the BHI changes was calculated using univariate and multivariate linear regression analysis. Multivariate linear regression analysis was done using forward stepwise approach to minimize the number of variables in the model because of a small sample size. Statistical significance was considered at $p<0.05$. The study was approved by the local ethic committee and each subject gave Informed consent.

Results

We studied 50 DM2 patients (33 men and 17 women) and 50 sex- and age-matched healthy controls (26 men and 24 women). The clinical and laboratory characteristics of the DM2 patients are presented in Table 1. Cardiorespiratory and MFV values with CO_2 change are shown in Table 2. The two groups had comparable cardiorespiratory values under resting and hypercapnia. Among the DM2 patients, 27/50 had hyperlipoproteinaemia, 11/50 atrial fibrillation, 40/50 hypertension, 7/50 mild stenosis of the main neck blood vessels, 12/50 were obese, 10/50 smoked and 7/50 consumed alcohol. Concerning the diabetic complications, 10/50 had retinopathy, 6/50 nephropathy, 8/50 polyneuropathy and 8/50 previous neurological disturbances. We found statistically significant difference for the BHI between patients with the DM2 (BHI=0.69±0.31) and healthy controls (BHI=1.33±0.28, $p<0.001$). As we found a significant correlation between the BHI and age ($r=0.62$, $p<0.001$) within the healthy controls group, we made an adjustment for the BHI for age (BHI_{adj}) prior to the further analyses. In the DM2 group the BHI_{adj} was, in the univariate analysis, significantly associated with age ($p<0.001$), fasting glycemia ($p=0.04$) and albuminuria ($p=0.04$). We found no significant effects of other major vascular risk factors, previous neurological disturbances and diabetic complications in the DM2 patients on the BHI_{adj} ($p>0.05$ for all). Using a multivariate analysis, the BHI_{adj} was significantly associated with age, creatinine clearance and fasting glycemia (r for the model=0.64, $p<0.0001$) in the DM2 group (Table 3).

TABLE 2
CARDIORESPIRATORY AND MFV VALUES WITH CO_2 CHANGE

	At rest	Breath-hoding test
MFV dm (cm/s)	49±13.7	60±18
MFV control (cm/s)	65.3±7.4	90.8±11.8
SBP dm (mmHg)	130.1±6.1	133±7
SBP control (mmHg)	120±10	122±8
DBP dm (mmHg)	75±3	76±4
DBP control (mmHg)	70±4	72±4
Heart rate dm (beats/min)	66±4	67±2
Heart rate control (beats/min)	66±4	67±2
SaO_2 dm (%)	97.8±0.3	98.2±0.4
SO_2 control (%)	98.2±0.4	99.1±0.3

Definition of abbreviations: MFV – mean flow velocity, SBP – systolic blood pressure, DBP – diastolic blood pressure, SaO_2 – oxigen saturation

TABLE 3
MULTIVARIATE REGRESSION ANALYSIS OF LABORATORY PARAMETERS AND BHI_{adj}

Laboratory parameters	β	p-level
Age	0.52	<0.001
Creatinine clearance	0.32	0.007
Fasting glycemia	–0.25	0.030

Discussion and Conclusion

Acute stroke is still the leading cause of death and disability in modern society. In order to reduce incidence of acute stroke the aim of our study was to define some other risk factors for its development. Scientific attention is still more focused on the extracranial macrovascular complications of the diabetes mellitus than functional changes in cerebral small vessels. We try to identify early enough moderate or significant stenosis of the main neck blood vessels and then treat or observe them as stroke prevention. Moreover, focus of our interest was structural and functional changes in the cerebral small vessels, signs of silent cerebral microangiopathy. From all known methods which can assess the CVR, functional TCD with the BHT was the most pleasant, easiest, noninvasive, reproducible, real-time and bedside method. As it was mentioned before, the fall in the cerebral perfusion pressure have no influence on regional cerebral blood flow in the healthy individuals because of the cerebral auto-regulation. These compensatory mechanisms can be exhausted in some pathological conditions[6,7]. Results of our study showed that CVR was impaired and cerebral autoregulation was exhausted in the DM2 patients. CVR was in relation with age, albuminuria, creatinine clearance and fasting glycemia. In a group of healthy controls we found significant correlation between CVR and age, like it was found in some previous studie[24,25] and we made correction of value in the DM2 group, prior to the further analyses. As we know, age is an independent risk factor for stroke due to remodeling of the cerebral small vessels[26]. After correction, we still found correlation between age and CVR in the DM2 patients. A large epidemiological study showed that age and previous stroke are the main predictors of a stroke in the DM2[27]. Among patients without a history of stroke, HbA1c and smoking were predictors of the stroke and microvascular complications. In the group of patient with his-

tory of the stroke, predictors of the stroke were increased total cholesterol and low HDL cholesterol concentration[27]. We found no correlation between CVR and mentioned risk factors. A decreased CVR indicates the presence of the preexisting vasodilatation, which reflects a reduced reserve capacity of the cerebral autoregulation[16] and an increased risk for stroke[6–8,28]. Intracerebral arterioles could be already maximally dilated and unable to react to stimuli[16]. Although vasodilatatory ability of brain arterioles decreased with increasing disease duration[19–22], we did not find any correlation between them. Decreased CVR may be responsible for a worse outcome of strokes in the diabetic patients[19]. As we know from previous studies, hyperglycemia leads to impaired vascular function through endothelial cell dysfunction and the HbA1c is an indicator of the severity of diabetic cerebral microangiopathy[20,21], but we did not find any correlation between the HbA1c and CVR in our patients. Poor glycaemic control in the DM2 increases cardiovascular risk[29]. In a large cohort study, fasting plasma glucose was found to be a predictor for stroke in patients with the DM2[29]. Furthermore, fasting plasma glucose (FPG) was associated with incident ischemic cerebrovascular in increase DM2 patients with FPG concentration above 5,6 mmol/L[30]. We found correlation between the CVR and fasting glycaemia, which was in accordance with results of mentioned studies. Other published studies did not find correlation between fasting glucose and CVR in DM2 patients[19–22].

In our study, a significant correlation between BHI_{adj} and both creatinine clearance and albuminuria was found in DM2 patients. However, some studies[19,22] failed to find correlation between CVR and albuminuria, like we did. Although, albuminuria is consider as an independent predictor of increasing levels of vascular risk factors and macroangiopathic carotid alteration and microvascular brain as it was confirmed by several studies[31,32]. Correlation between albuminuria, creatinine clearance and BHI, confirmed by our study, is in accordance with earlier observations that presence of renal microangiopathic changes can indicate existing cerebral microangiopathic changes and reverse too[21]. Our observation showed that CVR was not associated, like in other cases[19–22], with presence of the additional risk factors, existing any diabetic complication, inflammation factors, hepatic enzymes, waist measurement. The limitation of our study is that there were a small number of patients and controls. We present now our preliminary results and continue with study. In conclusion, the CVR is impaired and cerebral autoregulation is exhausted in patients with the DM2. Results of our study showed that age, fasting glycemia and albuminuria were in correlation with BHI in DM2 patients. These results may carry important implications for the prevention strategies in treatment of DM2 patients. Certainly, the CVR has to be regularly evaluated and followed up for early therapeutical reaction and prevention decompensation of the intracranial circulation and stroke. The TCD is the most accurate method in the evaluation and monitoring of the cerebral haemodynamic changes in the diabetic microangiopathy, especially silent ones, and has to be performed periodically on a routine basis in the diabetic patients. In order to carefully evaluate CVR and cerebral autoregulation in DM2 patients and to reduce the risk of stroke, further studies in a greater number of patients are needed.

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OSLABLJENA MOŽDANA VAZOREAKTIVNOST KOD BOLESNIKA SA ŠEĆERNOM BOLESTI TIP 2

S A Ž E T A K

Cilj ovog istraživanja bio je procijeniti moždanu vazoreaktivnost kod bolesnika sa šećernom bolesti tip 2 (DM2), te faktore koji na nju utječu. Prema dosadašnjim studijama koje su slično procjenjivale moždanu vazoreaktivnost kod DM2, rezultati su dvojbeni. Procjenu stanja moždane vazoreaktivnosti smo radili mjerenjem indeksa zadržavanja daha (BHI) koristeći Transkranijalni dopler (TCD) krvnih žila na 50 bolesnika sa DM2 i 50 zdravih, po dobi i spolu odgovarajućih kontrola. Promatrani su uz epidemiološke i kliničke podatke i ostali vaskularni rizični faktori te laboratorijski parametri. Nađena je statistički značajna razlika u indeksu zadržavanja daha između bolesnika sa DM2 (BHI= 0,69±0,31) i zdravih ispitanika (BHI=1,33±0,28, $p<0,05$). Zbog nađene značajne korelacije BHI s dobi ($p<0,001$) kod zdravih, rađena je korekcija vrijednosti u dijabetičara za dob prije daljnje analize (BHIadj). U skupini DM2 nađena je korelacija BHI osim sa dobi ($p<0,001$) i sa GUK-om natašte ($p=0,04$) i albuminurijom ($p=0,04$), te u multivarijantnoj analizi s kreatinin klirensom. Naše istraživanje je pokazalo da je kod bolesnika sa DM2 oslabljena cerebrovaskularna reaktivnost, a stupanj oštećenje je povezan sa dobi, GUK-om natašte i renalnom funkcijom. Funkcionalni TCD je kod njih dobra skrining metoda za otkrivanje i praćenje cerebralnih mikroangiopatskih promjena.