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Cerebral vasoreactivity in hypocapnia and hypercapnia in patients with diabetes mellitus type 2 with or without arterial hypertension

Reaktywność naczyń mózgowych w warunkach hipokapnii i hiperkapnii u chorych na cukrzycę typu 2 z nadciśnieniem tętniczym i bez nadciśnienia tętniczego

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Abstract

Background and purpose: Diabetes mellitus (DM) is an independent risk factor for cardiovascular diseases. The origin of diabetic microangiopathy is multifactorial; it affects all layers of the artery wall, causing endothelial and vasoreactivity impairment. The incidence of cerebral vasoreactivity failure in diabetic patients without stroke history is not precisely determined yet. The aim of the study was to assess the cerebrovascular reactivity in hypocapnia and hypercapnia in patients with type 2 DM with or without arterial hypertension without artery stenosis and stroke history, with the use of transcranial Doppler examination.

Material and methods: The mean blood flow velocity, pulsatility index and parameters of cerebrovascular reactivity were measured in 53 patients with type 2 DM (aged 42-72 years, mean 59.5 ± 7.9) and in 27 healthy volunteers (aged 36-74 years, mean 57.0 ± 10.4). Diabetics were further divided into two subgroups according to the presence or absence of arterial hypertension.

Results: The index of cerebrovascular reactivity in hypocapnia and hypercapnia was significantly worse and time needed to normalization of blood flow velocity was significantly longer in patients with DM in comparison with healthy volunteers.

Streszczenie

Wstęp i cel pracy: Cukrzyca to niezależny czynnik ryzyka rozwoju chorób układu naczyniowego. Pochodzenie cukrzycowej angiopatii jest wieloczynnikowe. Nie określono dotąd dokładnie częstości występowania upośledzenia reakcji wazoaktywnych u chorych na cukrzycę typu 2 bez incydentów udarowych w wywiadzie. Celem pracy było zbadanie przy użyciu przezczaszkowej ultrasonografii prędkości średniej przepływu krwi, współczynnika pulsacji oraz parametrów reaktywności naczyń mózgowych w warunkach hipokapnii i hiperkapnii u chorych na cukrzycę typu 2 z nadciśnieniem tętniczym lub bez nadciśnienia tętniczego bez ultrasonograficznych cech zwężeń tętnic szyjnych i mózgowych oraz nieobciążonych udarem mózgu.

Materiał i metody: Badania wykonano u 53 chorych (27 kobiet i 26 mężczyzn) w wieku od 42 do 72 lat (średnia wieku 59,5 ± 7,9 roku) z cukrzycą typu 2. Wśród chorych wydzielono podgrupy z nadciśnieniem tętniczym i bez nadciśnienia. Grupę kontrolną stanowiło 27 ochotników w wieku 36–74 lat (średnia wieku 57,0 ± 10,4 roku). U wszystkich przeprowadzono badanie dopplerowskie tętnic szyjnych i mózgowych oraz oznaczono współczynnik reaktywności naczyń mózgowych w warunkach hipokapnii i hiperkapnii.

Wyniki: W hipokapnii i hiperkapnii u chorych na cukrzycę typu 2 stwierdzono istotnie mniejsze współczynniki reaktywności naczyń mózgowych oraz znamiennie dłuższy czas

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Conclusions: Most DM type 2 patients without stroke history had decreased values of cerebral vasoreactivity parameters, which suggests the presence of microangiopathy.

Key words: cerebral vasoreactivity, diabetes mellitus, hypocapnia, hypercapnia.

normalizacji prędkości przepływu krwi w prawej tętnicy środkowej mózgu w stosunku do grupy kontrolnej.

Wnioski: U znacznej części chorych na cukrzycę typu 2 bez incydentów niedokrwienia mózgu w wywiadzie występują w hipokapnii i hiperkapnii mniejsze wartości parametrów reaktywności naczyń mózgowych, mogące sugerować obecność mikroangiopatii.

Słowa kluczowe: reaktywność naczyń mózgowych, cukrzyca, hipokapnia, hiperkapnia.

Introduction

Cerebral microangiopathy is one of the major causes of cerebrovascular events in patients with type 2 diabetes mellitus (DM). The small arteries in those patients feature thickened basement membrane, proliferation of endothelial cells, and abnormal collagen structure within the tunica externa [1].

The origin of diabetic angiopathy is multifactorial and should be viewed in the context of the changes in gene expression, hormonal and metabolic abnormalities, coexistent insulin resistance and the predisposition to arterial hypertension [2]. The early stages of microangiopathy include a period of structural adaptation and remodelling of the vessel wall, followed by damage to all layers of the vessel wall, including endothelium.

Changes in the architecture of the walls both in large and small cerebral arteries in DM suggest the presence of disordered reactivity of those vessels as the result of endothelial and tunica media dysfunction.

Increased or decreased blood flow velocities within the arteries at the base of the brain, as well as the changes in vessel reactivity, as recorded with the ultrasound Doppler study, might be considered as an index of the occurrence of diabetic microangiopathy [3-6]. Even in the early stages of the disease, capillaries show functional changes, i.e. increased blood flow, increased intravascular pressure, increased permeability and endothelial dysfunction [2,7]. Abnormalities of cerebral vessel reactivity might be important for the increased risk of cerebrovascular events in diabetic patients; they could also serve as an indicator of the severity of changes within the cerebral arteries [8-11].

The aim of the study was to assess the cerebrovascular reactivity indexes in hypocapnia and hypercapnia among patients with type 2 DM with or without arterial hypertension, who had neither carotid or coronary artery stenosis nor stroke history, i.e. in patients without clinical manifestation of small vessel disease.

Material and methods

This study initially recruited 95 subjects but 15 of them were excluded because of the poor transparency of the ultrasonographic temporal bone windows, due to stenosis within the carotid arteries or due to significant (> 15%) asymmetry of the blood flow velocity between the respective arteries on both sides.

Finally, the study was conducted in 53 patients (27 women and 26 men), aged between 42 and 72 (mean 59.5 ± 7.9) and diagnosed with type 2 DM according to the criteria proposed by the American Diabetes Association (1997) and by the World Health Organization (WHO, 1999).

Twenty-one patients (40%) had arterial hypertension. All diabetic patients (mean disease duration 7 ± 1.6 years) had good glycaemic control, as suggested by the daily measurements of fasting and postprandial blood glucose registered in 3-month diaries.

Inclusion criteria were: (1) diagnosis of type 2 DM, (2) no current or previous symptoms or signs suggestive of focal brain damage (stroke, transient ischaemic attack, reversible ischaemic neurological deficit); (3) no current or previous serious head trauma or any other disease of the central nervous system; (4) age between 35 and 75 years; and (5) lack of significant technical difficulties during the testing.

We excluded patients who: (1) had stenosis or occlusion of carotid or intracranial arteries in Doppler study; (2) had migraine or other vascular headaches; (3) chronically used non-steroid antiinflammatory drugs, nitrates, or hormonal replacement therapy; (4) had lesion(s) typical for ischaemic lesions in magnetic resonance imaging (MRI), except for the lacunae; (5) were smokers; (6) had altered consciousness; (7) reported maximal values of systolic blood pressure > 200 mm Hg or diastolic blood pressure > 100 mm Hg; (8) were diagnosed with respiratory, renal, heart or liver failure; (9) were addicted to alcohol.

Participants were instructed neither to drink coffee, nor to take their regular medications, except for hypotensive drugs.

The control group consisted of 27 non-smoking volunteers (12 women and 15 men), aged between 36 and 74 (mean 57.0 ± 10.4) with back pain syndromes but without a history of DM, arterial hypertension, other cardiovascular diseases or cerebrovascular events. Mean age of patients and controls was similar ($p = 0.236$).

All participants were informed about the aim of the study and its procedures. They gave informed consent to participate. The study protocol was accepted by the Bioethical Committee at the Silesian Medical University of Katowice (no. NN-013-215/03).

Before the Doppler study, blood pressure and heart rate were measured. Further procedures were only performed in patients whose systolic blood pressure was 120-135 mm Hg, diastolic blood pressure was 70-85 mm Hg, and heart rate was 68-80 per minute.

During the first part of the procedure, mean blood flow velocity (V_{mean}) expressed in centimetres per second, as well as the Gosling pulsatility index (PI), were measured in common, internal and external carotid arteries (CCA, ICA and ECA, respectively), vertebral, basilar, anterior cerebral, middle cerebral and posterior cerebral arteries. Measurements were made in the supine position, except for the vertebrobasilar system assessment which was made in a sitting position. Blood flow parameters were assessed according to the criteria proposed by Demchuk *et al*. [12,13]. Interhemispheric differences for V_{mean} and PI did not exceed 15%.

The measurements were made once with the Pioneer TC 2020 (EME) apparatus with pulsating probes of 2 and 4 MHz. All procedures were performed in the morning, by the same investigator, which guaranteed the reproducibility of the results (intraobserver agreement was 0.73 in the group of 40 persons) (MedCalc Statistical Software v.12.1.1). So-called blind Doppler was used rather than the duplex examination to detect possible significant stenoses that might have significantly affected the blood flow in cerebral arteries.

The second stage of the examination involved evaluation of the cerebral vasoreactivity index during hypocapnia and hypercapnia, using previously published me - thodological standards [14-17].

The measurements of vasoreactivity were performed within the right middle cerebral artery, as no significant difference of blood flow velocity was found between sides.

Vasoreactivity indexes were calculated from the equation proposed by Kastrup and adapted for hypocapnia and hypercapnia conditions [14]:

$$\text{Reactivity index} = \frac{(V_{mean\ N} - V_{mean\ H}) \times 100\%}{V_{mean\ N} \times (\Delta pCO_2)\ [\%/\text{mm Hg}]}$$

Where $V_{mean\ N}$ indicates mean velocity within the right middle cerebral artery in normocapnia, $V_{mean\ H}$ indicates mean velocity within the right middle cerebral artery in either hyper- or hypocapnia, and ΔpCO_2 is the difference between end-tidal partial pressure of CO_2 in hypo/hypercapnia and normocapnia conditions.

Hypocapnia was induced by 3-minute hyperventilation following 3-minute resting. The measurements of end-tidal CO_2 partial pressure and the level of arterial blood saturation were made with the capnograph. Hypocapnia was defined as the end-tidal CO_2 partial pressure of 15-30 mm Hg corresponding to the same partial pressure interval in blood. The cerebrovascular reactivity index was assessed in the third minute of hyperventilation.

After a 3-minute rest period, hypercapnia was induced with 2-minute ventilation in a closed circuit with gas containing 95% air and 5% CO_2 given through an anaesthetic mask connected with the gas reservoir. Hypercapnia was defined as the end-tidal CO_2 partial pressure of 45-60 mm Hg corresponding to the same partial pressure interval in blood. The cerebrovascular reactivity index was assessed in the first minute after the 2-minute ventilation with the gas of increased CO_2 concentration.

End-tidal CO_2 partial pressure was assessed with a capnograph (COSMO 2, Novametrix) with the sensor mounted on the special tube used by the participants for breathing with closed nostrils. Pulse oximetry was used to measure the saturation of the arterial blood. Blood flow parameters were calculated as means of the readings made within three cardiac cycles.

Additionally, the time needed for the normalization of the V_{mean} obtained in hypo- or hypercapnia was calculated.

Mean 3-month glycaemia was calculated using the data stored in diaries completed by diabetic patients. It was similar between patients with and without arterial hypertension.

Descriptive statistics (means, medians, quartiles, standard deviations) and the analysis of statistical significance of differences were performed with STATISTICA v.7 PL (StatSoft). Distribution of each analysed variable was tested with the Shapiro-Wilk test. As those distributions deviated significantly from the normal one, the non-parametric Mann-Whitney *U*-test was used for comparisons between groups. *P*-values of less than 0.05 were considered statistically significant.

Results

Normocapnia conditions

Blood flow velocity in the right middle cerebral artery in normocapnia (median, 48 [interquartile range, 42-54] cm/s) in patients with type 2 DM did not differ from that in controls (51 [48-55] cm/s). It was also similar in the subgroup of diabetic patients without arterial hypertension (50 [43-56] cm/s) and in diabetic patients with arterial hypertension (48 [42-50] cm/s).

PI in diabetic patients without hypertension (0.97 [0.83-1.05]) and in diabetics with hypertension (0.99 [0.79-1.05]) was significantly higher than in controls (0.85 [0.78-0.98], $p = 0.041$ and 0.0205, respectively).

Table 1. Comparison of blood flow parameters in right middle cerebral artery in patients with type 2 diabetes mellitus without arterial hypertension with the control group in hypo- and hypercapnia (values are presented as medians with interquartile range)

Variable	Patients with diabetes mellitus	Controls	*P*-value
Mean velocity [cm/s]			
Hypocapnia	35.5 (28.0-38.5)	33.0 (30.0-36.0)	NS
Hypercapnia	65.5 (56.5-76.0)	73.0 (70.0-80.0)	0.0446
Pulsatility index			
Hypocapnia	1.30 (1.19-1.62)	1.20 (0.90-1.50)	NS
Hypercapnia	0.77 (0.69-0.87)	0.71 (0.68-0.80)	NS
Cerebral vasoreactivity index [%/mm Hg]			
Hypocapnia	2.58 (2.08-2.90)	3.53 (3.27-3.78)	0.0000
Hypercapnia	3.43 (2.58-4.07)	4.50 (3.74-5.21)	0.0002
Time needed for parameters' normalization [s]			
Hypocapnia	2.00 (1.43-2.65)	1.20 (0.90-1.50)	0.0004
Hypercapnia	0.50 (0.38-0.82)	0.25 (0.25-0.35)	0.0000

NS – non-significant

Table 2. Comparison of blood flow parameters in right middle cerebral artery in patients with diabetes mellitus and arterial hypertension with the control group in hypo- and hypercapnia (values are presented as medians with interquartile range)

Variable	Patients with diabetes mellitus and arterial hypertension	Controls	*P*-value
Mean velocity [cm/s]			
Hypocapnia	33.0 (31.0-38.0)	33.0 (30.0-36.0)	NS
Hypercapnia	61.0 (53.0-71.0)	73.0 (70.0-80.0)	0.0063
Pulsatility index			
Hypocapnia	1.39 (1.27-1.64)	1.20 (0.90-1.50)	0.0205
Hypercapnia	0.78 (0.68-0.84)	0.71 (0.68-0.80)	NS
Cerebral vasoreactivity index [%/mm Hg]			
Hypocapnia	2.50 (1.90-2.90)	3.53 (3.27-3.78)	0.0000
Hypercapnia	2.77 (1.78-3.86)	4.50 (3.74-5.21)	0.0000
Time needed for parameters' normalization [s]			
Hypocapnia	2.30 (2.10-3.00)	1.20 (0.90-1.50)	0.0000
Hypercapnia	0.60 (0.50-1.00)	0.25 (0.25-0.35)	0.0000

NS – non-significant

Hypocapnia conditions

Diabetic patients both with ($p = 0.0004$) and without arterial hypertension ($p < 0.0001$) had longer time needed for normalization of the blood flow velocity in the right middle cerebral artery in comparison to controls. The cerebrovascular reactivity index was lower in diabetic patients (with or without arterial hypertension) than in the control group ($p = 0.0000$). Blood flood velocity in the right middle cerebral artery, PI and cerebrovascular reactivity indexes were all within the normal range in patients and controls (Tables 1 and 2).

Normal values of the cerebrovascular reactivity index found in this study in hypocapnia conditions were between 3.27 and 3.78%/mm Hg (interquartile range).

Hypercapnia conditions

Blood flow velocity in the right middle cerebral artery was lower in diabetic patients with hypertension ($p = 0.0063$) or without hypertension ($p = 0.0446$) than in controls. Diabetic patients both with and without arterial hypertension had longer time needed for normalization of the blood flow velocity in the right middle cerebral artery in comparison to controls (both $p < 0.0001$). The cerebrovascular reactivity index was lower in diabetic patients (with or without arterial hypertension) than in the control group ($p < 0.0001$ and $p = 0.0002$, respectively). Blood flood velocity in the right middle cerebral artery, PI and cerebrovascular reactivity indexes were all within the normal range in patients and controls, similarly to the hypocapnia conditions (Tables 1 and 2).

Normal values of the cerebrovascular reactivity index found in this study in hypercapnia conditions were between 3.74 and 5.21%/mm Hg (interquartile range).

Discussion

Transcranial Doppler ultrasonography enables measurements of the blood flow velocity in cerebral arteries and the registration of blood flow changes during provocative tests. It helps therefore to assess the ability of cerebral vessels to react to the changing partial pressures of CO_2. According to the experimental studies, decreased reactivity suggests abnormalities within the vessel wall [3].

The goal of the present study was to assess the cerebrovascular reactivity changes in patients with type 2 DM before the clinical manifestation of the focal brain ischaemia.

About 40% of studied patients had arterial hypertension, which itself predisposes to the microangiopathic changes. Patients with type 2 DM were therefore divided into two subgroups: those with and without arterial hypertension.

Diabetic patients without arterial hypertension had higher PI values than healthy controls. PI increases in case of increased peripheral vascular resistance, locally before the site of major stenosis or occlusion, and generally in small vessel disorders [18,19].

Increased PI was noted in patients in whom the stenosis or occlusion of the extra- or intracranial arteries was *a priori* excluded. It therefore reflects increased vascular resistance due to the vessel wall remodelling in arterioles. Hence, these results may reflect the presence of abnormalities within the arteriole walls and suggest that type 2 DM might be an independent risk factor for the cerebral microangiopathy.

Lee *et al*. [18] found an increased mean PI value in the internal carotid artery and middle cerebral artery in patients with type 2 DM who had diabetic complications (retinopathy, nephropathy, neuropathy), while in diabetics without clinical manifestation of the microangiopathy, PI values were similar to those obtained in healthy subjects. Lippera *et al*. [3] used transcranial Doppler to study blood flow parameters in anterior and middle cerebral arteries, as well as in ophthalmic arteries, and also noted an increased PI in patients without a history of neurological events, who had microangiopathy in extracerebral vessels. Those authors suggested the existence of latent diabetic cerebral microangiopathy.

Hypocapnia physiologically leads to vasoconstriction which manifests with decreased blood flow velocity and increased PI. The attenuated vasoconstriction in response to hypocapnia is probably associated with the structural changes of the vessel wall that also result in dysfunction of endothelin-1 (ET-1) release, which is the major mediator of the vasoconstriction in hypocapnia [20].

Kanno *et al*. [21] and Smulders *et al*. [22] reported decreased serum ET-1 concentrations in patients with type 1 and type 2 DM. Similarly, Vazquez *et al.* [23] observed decreased ET-1 levels in patients with type 1 DM and with subclinical regional cerebral hypoperfusion, as assessed with SPECT.

Abnormal vasoconstrictive responses in DM might also be related to receptor dysregulation, altered sensitivity to CO_2, as well as to the lower compliance of the vessel wall. The role of the sympathetic innervation in direct and indirect regulation of muscle tone within the vessel wall should also be considered.

The present study also showed that patients with type 2 DM and without arterial hypertension additionally feature very slow normalization of the blood flow parameters after the resolution of hypocapnia. This finding has not been reported previously, and the mechanism of such a response is unclear. It is possible that this blunted response is due to the insufficient compensatory mechanism that reverses the decreased blood flow in hypocapnia, i.e. the action of the vasodilators. The attenuated vasoconstriction of the arterioles and the prolonged time of their return to baseline in patients with type 2 DM might suggest damage of the vessel wall, i.e. the presence of microangiopathy.

Hypercapnia physiologically leads to vasodilation, which manifests with increased blood flow velocity and decreased PI. We found that the increase of that velocity, as well as its final value, was lower in patients with type 2 DM and without arterial hypertension than in healthy subjects.

Dandona *et al*. [24] and Fulesdi *et al.* [25] observed a smaller increase of the average mean velocity in hypercapnia and decreased parameters of vascular reactivity in patients with type 1 DM, while Tantucci *et al*. [26] reported similar observations in patients both with type 1 and with type 2 DM. The authors highlight the role of autonomic neuropathy in the attenuation of the vasodilatory response to increased partial pressure of CO_2. Decreased reactivity index values in patients with type 1 DM were also reported by Kozera *et al*. [27].

Our patients with type 2 DM, when compared to the healthy subjects, had significantly prolonged time needed for normalization of parameters after hypercapnia. Its mechanism is also unclear but it possibly results from the abnormal compensation reactions, similarly to the same finding in hypocapnia.

Our findings support the presence of attenuated vasoactive responses to the chemical stimulus in patients with type 2 DM, which may suggest that the disease is an independent factor influencing the ability of cerebral arteries to constrict and to dilate.

Given the fact that the attenuated vasoactive responses might reflect structural or humoral insufficiency of the cerebral arterioles, it may be suspected that subclinical cerebral microangiopathy is quite prevalent among patients with type 2 DM. Blood flow parameters among the studied diabetic patients were within the normal range in the majority of cases, although they differ significantly from those obtained in the healthy subjects. It is worth noting that those patients had neither previous clinical episodes of brain ischaemia nor signs of focal damage to the central nervous system.

Attenuated reactivity of the cerebral vessels in those patients raises the suspicion that despite the lack of signs of central nervous system damage, some of those patients may actually have a dysfunction of the cerebral arterioles, which probably reflects damage of the vessel wall and points to the risk of clinical events of cerebral ischaemia.

Limitations of the present study include the relatively small study sample, which resulted from the difficulties in recruitment of diabetic patients who fulfil the other inclusion criteria. The other limitation was the single examination of cerebrovascular reactivity; multiple examinations with the averaging of results might better reflect the values of the studied parameters.

Conclusions

The majority of DM type 2 patients without stroke history had decreased values of cerebral vasoreactivity parameters, which suggests the presence of microangiopathy.

Disclosure

Authors report no conflict of interest.

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