Serum 25-hydroxyvitamin D levels in stroke patients with and without carbohydrate metabolism disturbances in north-eastern Poland

Stężenie 25-hydroksywitaminy D w surowicy u pacjentów z udarem, z zaburzeniami i bez zaburzeń w metabolizmie węglowodanów, w północno-wschodniej części Polski

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Wprowadzenie. Plejotropowe działanie witaminy D w organizmie człowieka obejmuje kontrolę wielu procesów fizjologicznych i patofizjologicznych. Jednym z nich jest ochrona komórek nerwowych podczas udaru, jak również kontrola metaboliczna i udział w patogenezie cukrzycy typu 2. Stężenie w surowicy krwi 25-hydroksywitaminy D (25(OH)D) poniżej 20 ng/ml wskazuje na niedobór witaminy D, co jest powszechne w Polsce.

Cel. Ocena stężenia 25-hydroksywitaminy D w surowicy krwi, w czasie ostrego udaru niedokrwiennego mózgu, u pacjentów z zaburzeniami i bez zaburzeń w metabolizmie węglowodanów.

Materiały i metody. Badaniami objęto 59 pacjentów z ostrym udarem niedokrwiennym mózgu hospitalizowanych w Klinice Neurologii w 2012 r. (36 z zaburzeniami w metabolizmie węglowodanów i 23 bez cukrzycy i stanu przedcukrzycowego) oraz 10 osób, jako grupa kontrolna. U pacjentów przeprowadzono badanie kliniczne oraz pobrano krew celem oceny stężenia w surowicy: 25(OH)D, glukozy na czczo, HbA1c, lipidów, kreatyniny, wapnia i fosforu.

Wyniki. Średni wiek pacjentów wyniósł 66,1 lat. Średnie stężenie 25(OH)D w całej grupie badanej było bardzo niskie (8,1±6,8 ng/ml); w grupie kontrolnej: 7,1±6,7 ng/ml; średnie stężenie u pacjentów z udarem i z zaburzeniami w metabolizmie węglowodanów wynosiło 8,6±6,1 ng/ml, a u pacjentów bez zaburzeń w metabolizmie węglowodanów 7,2±7,7 ng/ml. Nie zaobserwowano istotnej różnicy w stężeniu 25(OH)D pomiędzy badanymi grupami (p=0,43). W całej badanej grupie u co drugiego badanego stężenie wapnia w surowicy było poniżej normy.

Wnioski. U pacjentów z ostrym udarem niedokrwiennym mózgu stężenie 25(OH)D jest bardzo niskie, zarówno u chorych z zaburzeniami w metabolizmie węglowodanów, jak i bez. W związku z tym należy prowadzić suplementację witaminą D w tej grupie pacjentów.

Słowa kluczowe: *witamina D, udar, cukrzyca*

Introduction. The pleiotropic effects of vitamin D extend to control many physiological and pathophysiological processes in the human body. One of them is protection of nerve cells during stroke, as well as metabolic control and participation in the pathogenesis of type 2 diabetes. The concentration of serum 25-hydroxyvitamin D (25(OH)D) lower than 20 ng/ml indicates vitamin D deficiency, and is common in Poland.

Aim. To estimate serum levels of 25(OH)D in stroke patients with and without carbohydrate metabolism disturbances.

Material & Method. We examined 59 patients with stroke admitted to the Department of Neurology in 2012 (36 with carbohydrate metabolism disturbances and 23 without diabetes or prediabetes), as well as 10 healthy control subjects. The clinical examination and blood samples were taken to assess serum 25(OH)D, fasting glucose, HbA1c, lipids, creatinine, calcium and phosphorus concentrations.

Results. The mean age of stroke participants was 66.1 years. The level of 25(OH)D in stroke patients in the entire group was 8.1±6.8 ng/ml, and without stroke 7.1±6.7 ng/ml. The level of 25(OH)D in patients with stroke and carbohydrate metabolism disturbances was 8.6±6.1 ng/ml, and with stroke and without carbohydrate metabolism disturbances was 7.2±7.7 ng/ml. The difference between the concentrations of 25(OH)D in the studied groups was insignificant (p=0.43). We observed the serum calcium concentration below the range norm in more than 50% of the stroke patients.

Conclusion. The patients with acute ischemic cerebral stroke, independently of carbohydrate metabolism disturbances, have low concentrations of 25(OH)D and should supplement this vitamin in the north-eastern part of Poland.

Key words: *vitamin D, stroke, diabetes*

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Introduction

The pleiotropic effects of vitamin D extend to control many physiological and pathophysiological processes in the human body. The vitamin D receptors have been found in most body tissues and are involved in the regulation of cellular proliferation, differentiation, apoptosis and angiogenesis [1]. The serum concentration of 25-hydroxyvitamin D (25(OH)D) below 20 ng/ml (<50 nmol/l) is considered indicative of the vitamin D deficiency, whereas the target concentration for optimal vitamin D effects is 30-50 ng/ml (75-125 nmol/l) [2]. Insufficient concentration of vitamin D is observed in populations of the northern and southern hemisphere [2, 3]. The problem seems to be crucial, because the 25(OH)D level below the normal range is considered to be a global health problem and is very common, also in Poland [2-6], and should be treated accordingly [2, 4].

It is known that stroke is the third leading cause of death in the world and very often leads to disability [7], whereas vitamin D has a protective function in the incidence of ischemic stroke and its severity [8, 9]. Poor vitamin D status could be associated not only with an increased stroke risk but also with adverse health problems in patients after stroke [10, 11]. It has been shown that low levels of vitamin D are associated with many risk factors of stroke, e.g. arterial hypertension, dyslipidemia, increased body mass index and waist-to-hip ratio or carotid intima-media thickness [12-14]. There are many theories explaining the vitamin D deficiency as the risk factor of stroke, especially ischemic stroke. First of all, some experimental studies have shown the antihypertensive and vascular protective effects of vitamin D, such as suppression of the renin-angiotensin-aldosterone system, lowering parathormone level, anti-atherosclerotic properties and the improvement of endothelial function [15]. Secondly, the vitamin D deficiency may increase the risk of infections, which could be a trigger factor of stroke [14]. Moreover, the vitamin D receptors have been identified in the brain and have neuroprotective properties [16] by affecting the expression of various neurotrophins and calcium-binding proteins that are crucial for normal brain function [17]. Low levels of vitamin D are also associated with decreased cognitive function in elderly people [18]. The above data are primarily observational studies, whereas randomized controlled trials (RCTs) are mixed. In an RCT performed by Hsia, et al. it was not confirmed that the calcium and vitamin D supplementation was connected with altered cerebrovascular risk in generally healthy postmenopausal women over a 7-year use period [19].

Type 2 diabetes is a global health problem and the vitamin D deficiency is common in this group of patients [20]. It has been shown that the vitamin D deficiency is associated with a substantial increase in the incidence of type 2 diabetes [21]. On the other hand, oral administration of a single large dose of vitamin D is connected with an improvement of endothelial function in patients with type 2 diabetes and the vitamin D deficiency [20]. Insufficient glycemic control may affect the serum 25(OH)D levels by different mechanisms. Firstly, worse glycemic control and poorer dietary habits could be indirectly associated with lower physical activity and lower exposure to sunlight [15]. Furthermore, it has been observed that correction of acute hyperglycemia causes an increase in serum concentrations of 25(OH)D and it was shown that hyperglycemia can disrupt the activity of 25-hydroxylase [22]. On the other hand, vitamin D may increase secretion of insulin by pancreatic β cells and improve the glucose and lipid metabolism in the skeletal muscle [5, 23-25]. In a recent meta-analysis of RTCs, Seida, et al. did not find any effect of the vitamin D supplementation on glucose homeostasis or diabetes prevention. However, conclusions drawn from this meta-analysis are restrained because the follow-up time was too short and the groups were not homogenous [26].

Thus, the vitamin D deficiency may play an important role in the development of both diseases, ischemic stroke and type 2 diabetes [1, 21]. At the same time, knowledge about the role of this deficiency is limited.

Aim

To estimate the serum levels of 25-hydroxyvitamin D in acute ischemic stroke patients with and without glucose metabolism disturbances.

Material and method

We examined 69 patients: 59 patients with acute ischemic cerebral stroke (24 females and 35 males) admitted to the Department of Neurology in 2012 and 10 subjects as a control group. Thirthy six studied patients presented glucose metabolism disturbances (GMD) and 23 patients did not have diabetes or prediabetes. The exclusion criteria from the current study were: recent acute illness, advanced chronic liver or renal diseases (stage 3 according to NKF), type 1 diabetes mellitus, previous immobility or failure to understand the protocol of the current study. The diagnosis of stroke was confirmed by computed tomography, as well as by clinical examination (based on clinical presentation, with the symptoms onset more than 24 hours earlier) according to the WHO criteria. The National Institute of Health Stroke Scale was used to assess the severity of stroke. Diabetes was defined as diagnosed earlier, oral hypoglycemic drugs/insulin use or by the WHO criteria. All the subjects gave written informed consent prior to entering the study. The study protocol was approved by the Ethics Committee, Poland (No R-I-002/573/2011).

Anthropometric measurements

Anthropometric parameters were measured in all subjects. BMI was calculated as body weight x height^{-2} and expressed in kg/m^2. Waist circumference was measured at the smallest circumference between the rib cage and the iliac crest.

Samples

Blood samples were collected from the subjects after an overnight fast, the day after admission to the hospital, in order to determine the concentrations of serum 25-hydroxyvitamin D, glycated hemoglobin (HbA1c), calcium, phosphorus, creatinine (glomerular filtration rate (GFR) was calculated), total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides (TG). The blood was frozen at -70°C until the estimation of 25(OH)D.

Biochemical analysis

25-hydroxyvitamin D was measured using a commercially available 25(OH)D direct ELISA Kit (ImmunDiagnostik AG, Bensheim, Austria) assayed with a microplate reader μQuant Biotek Instruments Inc. (Winooski, Vermont, USA). The coefficients of intra-assay and inter-assay variation were below 7%. Serum total cholesterol, HDL-cholesterol and TG were assessed by enzymatic methods (Cormay, Warsaw, Poland). Serum LDL-cholesterol was calculated from Friedewald's formula.

Statistical analysis

The statistical analyses were performed using the Statistica 10.0 program (StatSoft, Krakow, Poland). The differences between the groups were evaluated with an unpaired Student's t-test. The relationships between the variables were estimated with the simple analysis. The significance level was set to 0.05.

Results

The clinical characteristics of the subjects are shown in Table I. The mean age of the participants was 66.1 years (42-82). There were no significant differences in lipid concentrations or GFR between the studied groups (both $p>0.05$) (Tab. I). The mean level and SD (standard deviation) of 25-hydroxyvitamin D in the stroke patients in the entire group was 8.1±6.8 ng/ml, in the patients with carbohydrate metabolism disturbances it was 8.6±6.1 ng/ml, and in the patients without carbohydrate metabolism disturbances 7.2±7.7 ng/ml. The control group subjects also had very low concentrations of 25-hydroxyvitamin D: 7.1±6.7 ng/ml. The difference in concentrations of 25-hydroxyvitamin D between the studied groups was statistically insignificant (with vs. without glucose metabolism disturbances: $p=0.43$). None of the patients had sufficient vitamin D levels as compared to the recommended value (reference value in Poland 30-50 ng/ml) [2]. Moreover, fifty percent of the studied patients had calcium levels below the normal range in the entire group. We observed a negative correlation between fasting glucose levels and serum 25-hydroxyvitamin D concentration in patients with glucose metabolism disturbances ($r=-0.46$; $p=0.005$), whereas we did not find this correlation in the entire group ($r=-0.24$; $p=0.07$) or in the patients with normal glucose tolerance (NGT) ($r=-0.24$; $p=0.28$).

We did not observe any difference between the number of hospitalization days between the patients with stroke and GMD vs. the patients with stroke and NGT (13.7±6.4 vs. 16.1±9.4 days; respectively;

Table I. Clinical and biochemical characteristics of studied groups (M±SD)
Tabela I. Kliniczna i biochemiczna charakterystyka badanych grup (M±SD)

	Patients with acute ischemic cerebral stroke /Pacjenci z ostrym udarem niedokrwiennym mózgu		Control group /Grupa kontrolna (n=10)	p (GDM vs. NGT) /(ZMG vs. PTG)
	GMD/ ZMG (n=36)	NGT/ PTG (n=23)		
Gender (male/female) /Płeć (mężczyzna/kobieta)	22/14	13/10	4/6*	0.7
Age (years) /Wiek (lata)	67.1±9.9	64.4±12.2	66.2±6.5	0.3
BMI (kg/m²)	29.6±5.0	27.9±6.7	27.9±3.5	0.2
Waist circumference /Obwód talii (cm)	105.3±14.0	97.6±14.4	96.6±7.3	0.05
Smoking (Y/N) /Palenie tytoniu (T/N)	14/20	6/17	2/8	0.1
HbA1c [%]	6.3±1.4	5.6±0.3	5.5±0.3	0.02
Fasting glucose /Glukoza na czczo [mg/dl]	134.7±40.6	88.7±8.3	91.1±3.8	<0.0001
GFR [ml/min]	108.6±25.1	103.7±32.2	110.3±10.8	0.5
Total cholesterol /Cholesterol całkowity [mg/dl]	203±50	217±35	204±26	0.2
TG [mg/dl]	132±107	106±44	137±31	0.2
LDL-cholesterol /Cholesterol LDL [mg/dl]	128±45	151±35	127±27	0.05
HDL-cholesterol /Cholesterol HDL [mg/dl]	49±13	48±11	45±6.8	0.7

GMD – glucose metabolism disturbances /ZMG – zaburzenia w metabolizmie glukozy
NGT – normal glucose tolerance /PTG – prawidłowa tolerancja glukozy

p=0.25). Moreover, we did not observe any correlation between the serum vitamin D concentrations and the number of hospitalization days in the whole studied group (p=0.26; r=0.15), in the patients with stroke and GMD (p=0.69; r=-0.15), as well as in the patients with stroke and NGT (p=0.26; r=0.17).

Discussion

Our study showed that there was a deficiency of 25(OH)D in ischemic stroke patients with and without glucose metabolism disturbances, as well as in healthy control group. Moreover, the concentrations of vitamin D were below normal range in all studied subjects. Other researchers also found low values of 25(OH)D, but not in all investigated patients, as we reported. Poole et al. showed that 77% of stroke patients had vitamin D levels below 30 ng/ml [10]. They collected patients with hemorrhagic and ischemic stroke and did not divide the group by the diabetes status. We studied patients only with ischemic stroke without kidney and liver diseases or acute illnesses. In the present study, we observed mean levels of 25(OH)D concentration of about 8 ng/ml in the patients with stroke who had glucose metabolism disturbances, as well as without diabetes or condition of prediabetes. Daga, et al. demonstrated that 91.1% of the diabetic patients had the vitamin D deficiency. In their study, the vitamin D concentration in diabetic patients was 7.8±1.2 ng/ml, however, in the non-diabetic individuals it was 16.6±7.8 ng/ml [27], but they studied patients without stroke. Kuhn et al. found an increased risk of stroke in patients with the concentration of vitamin D below 12.5 ng/ml as compared to the subjects with levels ⩾20 ng/ml [28]. It could mean that the patients from our study could have higher risk of recurring stroke in the future, because low level of vitamin D is an independent risk factor for stroke [28, 29]. The mechanism of the effect of vitamin D deficiency on ischemic stroke is not fully explained. Vitamin D is connected with atherosclerosis in many ways, e.g. by decreasing the renin-angiotensin-aldosterone system, inflammation, coagulation, proliferation of vascular smooth muscle cells and cardiomyocytes [13]. Low levels of 25(OH)D influence the activity/expression of macrophages and lymphocytes in atherosclerotic plaques, thus promoting chronic inflammation in the arterial wall [30]. It was discovered that the vitamin D supplementation reduced serum levels of C-reactive protein, TNF-alpha, interleukin-6, endothelial adhesion molecules, tissue matrix metalloproteinases and increased interleukin-10 [30, 31], as well as increased the nitric oxide concentrations [32].

On the other hand, Skaaby, et al. did not find any correlation between low levels of vitamin D and the incidence of stroke in the large general population study [33]. It is well-known that the prevalence of vitamin D deficiency is very high among elderly adults [4]. The reason for low levels of vitamin D depends on sunlight exposure, synthesis, dietary intake and absorption of vitamin D, as well as adiposity. Profound deficiency of vitamin D in our patients may be the result of them residing in the north-eastern part of Poland.

Therefore, vitamin D could only reflect chronic illness or unhealthy behavior. Moreover, the vitamin D status could be associated with healthier lifestyle and higher socioeconomic status. In a meta-analysis, Bolland, et al. showed that the vitamin D supplementation with or without calcium reduces skeletal and non-skeletal outcomes only up to 15% [34]. The possible explanation of their findings may be connected with an insufficient supplementation of vitamin D or with the fact that the trial inclusion criteria were too broad and duration of application was too short.

Additionally, in our study we found a negative correlation between the fasting glucose levels and concentrations of vitamin D in patients with glucose metabolism disturbances. It is partly consistent with large cross-sectional studies which showed a positive association between the serum 25(OH)D levels and insulin sensitivity, and negative correlations with fasting hyperglycemia and with the elevated glycated hemoglobin levels [35, 36]. It was shown that the vitamin D and calcium deficiency may have a negative impact on glycemia, whereas combined supplementation with both nutrients may be beneficial in optimizing glucose metabolism [24]. Some researchers emphasized that an appropriate level of vitamin D was responsible for insulin secretion and insulin sensitivity [5]. Chiu, et al. confirmed a positive correlation of 25(OH)D levels with insulin sensitivity and a negative correlation of hypovitaminosis D with pancreatic beta cell function in healthy adults without diabetes or prediabetes [37]. Other researchers have shown that the supplementation of vitamin D improved insulin secretion in subjects with type 2 diabetes [24]. In contrast, other data indicated that the vitamin D supplementation in type 2 diabetic patients did not have an effect on glycemic outcomes, whereas it could improve insulin sensitivity among patients only with baseline glucose intolerance [38]. Vitamin D could support pancreatic beta cell function in many ways in type 2 diabetes. First of all, vitamin D could influence insulin secretion and pancreatic beta cell survival by impact on generation and effects on cytokines [5]. Secondly, $1,25(OH)_2D_3$ increases insulin secretion by binding to the pancreatic beta cell vitamin D receptor and changes calcium flux through the beta cell membrane [5, 37]. Additionally, low levels of vitamin D are responsible for secondary hyperparathyroidism, whereas parathormone may be responsible for inhibiting insulin synthesis and secretion [5]. Moreover, hypovitaminosis D is related to an impaired endothelial function in patients with type 2 diabetes [10], which was shown to improve after a single 100,000 IU oral

dose of vitamin D2 [20]. Interestingly, it is possible that vitamin D could reduce microvascular and macrovascular adverse outcomes in patients with diabetes even without an effect on glycemic control [31, 39].

It should be noted that the vitamin D deficiency is very common in stroke patients [8, 40, 41]. It seems that measurements of the 25(OH)D serum concentration in stroke patients are useful, especially in the north-eastern part of Poland. In clinical practice, we should remember about multiple health benefits of the vitamin D supplementation, especially in view of the fact that treatment with vitamin D is easy, safe and cheap. The optimal levels of vitamin D in type 2 diabetic patients particularly after stroke remain unknown. In the general practical guidelines, the target concentration of vitamin D for optimal effects is 30-50 ng/ml (75-125 nmol/l) in Central Europe for neonates, infants, children and adolescents, as well as in adults [2]. The compliance and effectiveness of the vitamin D treatment can be evaluated by measuring the 25(OH)D levels after three months of treatment [42]. It is worth mentioning that in some situations, especially in dehydrated elderly people or patients with renal insufficiency, too high doses of vitamin D could be dangerous.

Our study has some limitations, including the size of the sample and the fact that we did not take into account the seasonal variation in 25(OH)D concentration. Moreover, $1,25(OH)_2D_3$ and parathormone levels were not estimated in this study. This study is retrospective and we could not exclude the fact that low 25(OH)D levels are the consequence and not the cause of the disease.

Conclusion

The results obtained here indicate that, in spite of the guidelines, patients with acute ischemic cerebral stroke, with or without carbohydrate metabolism disturbances, have decreased mean serum concentrations of vitamin D in comparison to the reference range norm. Therefore, they should take this vitamin in the north-eastern part of Poland.

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