ORIGINAL PAPER/*ARTYKUŁ ORYGINALNY*

Concentration of 25(OH)D_3 and calcium and phosphorus metabolism in patients suffering from relapsing-remitting multiple sclerosis. A pilot study

Stężenie 25(OH)D_3 i gospodarka wapniowo-fosforanowa u chorych na postać nawracająco-zwalniającą stwardnienia rozsianego. Doniesienie wstępne

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Neurologia i Neurochirurgia Polska 2013; 47, 2: 126-130
DOI: 10.5114/ninp.2013.34730

Abstract

Background and purpose: The aim of this study was to evaluate the concentration of 25(OH)D_3 and the indices of calcium and phosphorus metabolism at different times of relapsing-remitting multiple sclerosis (MS). The relationships between the concentrations of 25(OH)D_3 and the indices of calcium-phosphate metabolism were determined, depending on the duration of disease and vitamin and unsaturated fatty acids supplementation.

Material and methods: Thirty patients (15 at the early stage and 15 at the advanced stage of MS) were enrolled. Each patient underwent neurological examination; neurological motor disability was defined according to the Expanded Disability Status Scale (EDSS). The results were compared to the values obtained in the control group (15 individuals) selected according to the age of subjects, their residence, ethnicity and gender.

Results: Significantly lower serum concentrations of 25(OH)D_3 in MS patients compared to the control group were found. MS patients at the advanced stage of the disease (duration of 5-6 years) had lower 25(OH)D_3 concentrations than patients at the early stage of MS. Among patients in an advanced stage of MS, significantly lower levels of 25(OH)D_3 were found in women compared to men with a similar level of physical disability.

Streszczenie

Wstęp i cel pracy: Celem pracy była ocena stężenia 25(OH)D_3 i wskaźników gospodarki wapniowo-fosforanowej w różnym okresie trwania postaci rzutowo-zwalniającej stwardnienia rozsianego (SR). Określono zależności stężenia 25(OH)D_3 i wskaźników gospodarki wapniowo-fosforanowej od przebiegu choroby oraz stosowanej suplementacji witaminami i nienasyconymi kwasami tłuszczowymi.

Materiał i metody: Badaniem objęto 30 chorych na SR (15 we wczesnym oraz 15 w zaawansowanym okresie choroby). U wszystkich przeprowadzono badanie neurologiczne oraz określono aktualny stan niepełnosprawności ruchowej według *Expanded Disability Status Scale*. Wyniki porównano z 15-osobową grupą kontrolną, dobraną stosownie do wieku badanych, ich miejsca zamieszkania, pochodzenia etnicznego oraz płci.

Wyniki: Stwierdzono istotnie mniejsze stężenie 25(OH)D_3 w surowicy chorych na SR w porównaniu z grupą kontrolną. U osób chorujących dłużej (5–6 lat) stężenia 25(OH)D_3 były mniejsze niż u osób we wczesnym okresie choroby. W grupie osób chorujących dłużej stężenie 25(OH)D_3 było wyraźnie mniejsze u kobiet w porównaniu z mężczyznami o podobnym stopniu niepełnosprawności ruchowej. Osoby chore stosowały suplementację 25(OH)D_3 i nienasyconych kwasów tłuszczowych częściej niż grupa kontrolna.

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Received: 22.02.2011; accepted: 5.06.2012

Conclusions: Lower serum concentrations of 25(OH)D_3 in MS patients compared to the control group were found. The lowest concentrations of 25(OH)D_3 were found in female patients aged 20-40 and in patients with a longer disease duration without substantial impairment of calcium-phosphate metabolism.

Key words: multiple sclerosis, 25(OH)D_3, dietary supplements.

Wnioski: Stwierdzono mniejsze stężenie 25(OH)D_3 w surowicy chorych na SR niż w grupie kontrolnej. Najmniejsze stężenie 25(OH)D_3 odnotowano u kobiet pomiędzy 20. a 40. rokiem życia i u osób dłużej chorujących bez istotnych zaburzeń gospodarki wapniowo-fosforanowej.

Słowa kluczowe: stwardnienie rozsiane, 25(OH)D_3, suplementy diety.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Its aetiology is not fully known, and the pathology includes demyelination with axonal damage and limited remyelination. The disease features CNS damage, which is multifocal and disseminated in time, with heterogeneous symptomatology and divergent clinical course [1,2]. The first symptoms of MS appear most commonly between the age of 20 and 30. The disease is more common in women (women-to-men ratio is about 2 : 1 in Europe) and among Caucasians. The pathogenesis of the disease is multifactorial and includes genetic, immunopathological and environmental factors interacting in a complex fashion [3-5].

Epidemiological studies suggest that MS is a disorder associated with a geographic region. Prevalence of MS increases with increasing latitude and decreases closer to the equator, which points to the possible association between the prevalence of MS and exposure to sunlight [6,7]. The protective effect of sunlight seems to be related to the protective action of 25(OH)D_3.

25(OH)D_3 plays an important role in regulation of calcium and phosphorus metabolism; it is involved in cell proliferation and differentiation, as well as in immunological phenomena. Studies that used peripheral blood lymphocytes showed an inhibitory effect of 25(OH)D_3 on the production of inflammatory mediators, e.g. interleukin (IL)-2, IL-6, tumour necrosis factor alpha (TNF-α), γ-interferon and the presence of nuclear vitamin D receptor (nVDR) in T lymphocytes, as well as its increased expression in lymphocytes treated with 25(OH)D_3. On the other hand, 25(OH)D_3 stimulates the synthesis of anti-inflammatory IL-10 [8-10].

Ultraviolet radiation intensity in northern regions is insufficient for the adequate synthesis of 25(OH)D_3 [11]. Its biological half-life reaches 19 days, and therefore spring and winter are seasons with the greatest deficit of 25(OH)D_3. According to population studies, people living in different latitudes have different concentrations of 25(OH)D_3. These differences are also associated with sex and age. The greatest deficit of 25(OH)D_3 was noted among elderly people in northern parts of Europe and America. Women aged between 15 and 45 are at greatest risk of 25(OH)D_3 deficiency in combination with seasonal variations, and the onset of MS peaks between the third and fourth decade [12].

Because of its geographical location, Poland ranks among countries with high prevalence of MS. The estimated annual incidence of MS in Poland is 1.5-3.7 per 100 000 [13]. An assessment of calcium-phosphorus metabolism, as well as 25(OH)D_3 concentration, is important in complex medical care of these patients.

The aim of this study was to evaluate the concentration of 25(OH)D_3 and the indices of calcium and phosphorus metabolism at different stages of relapsing-remitting MS. We also evaluated the relationship between 25(OH)D_3 concentration or indices of calcium and phosphorus metabolism and the disease course or supplementation with vitamins or unsaturated fatty acids.

Material and methods

The study comprised patients with early or advanced relapsing-remitting MS (RRMS) diagnosed according to McDonald criteria. Patients were selected using the medical records of an out-patient neurological clinic or medical records of in-patients hospitalized between 2009 and 2010 in the Department of Neurology in Zabrze.

The control group consisted of 15 subjects matched with patients regarding age, place of residence, ethnicity (Caucasians) and occupation.

The study was carried out among 30 patients, including 15 patients in an early stage of MS (up to 6 months after the onset of symptoms) and 15 patients in an advanced stage of MS (5-6 years after the onset of symptoms).

Patients were included if their score in the Kurtzke Expanded Disability Status Scale was between 0.5 and

3.0. The mean number of relapses in patients with early RRMS was 2.4 and in patients with advanced RRMS it was 3.5. Those relapses were treated with corticosteroids. Blood for laboratory studies was drawn 4 ± 2 months (patients with early RRMS) or 8 ± 3 months (patients with advanced RRMS) after the end of the therapy with corticosteroids. Patients received neither immunomodulatory nor immunosuppressive treatment.

All study participants (patients and controls) lived in the Silesian voivodship and worked in a similar environment. Control subjects did not have any symptoms or signs of CNS damage; they did not exhibit any signs of systemic or metabolic disorders.

Serum concentrations of $25(OH)D_3$ (range: 11.1-42.9 ng/mL or 20.0-125.0 nmol/L) and parathormone (range: 15-65 pg/mL) were measured with a COBAS e 601 analyzer (Roche).

Serum phosphorus (0.81-1.45 mmol/L), calcium (2.1-2.55 mmol/L), ALP2L alkaline phosphatase (30-90 IU/L), bone alkaline phosphatase (20-48 IU/L), as well as daily urine excretion of calcium (2.5-6.5 mg/24 hours) were measured with a COBAS c 501 analyzer (Roche). Serum ionized calcium concentration (reference range: 1.13-1.32 mmol/L) was measured directly, using ion selective electrodes (RAPID lab 865, Siemens).

The following meteorological data were obtained from the Institute of Meteorology and Water Management, Upper Silesian Hydrological and Meteorological Centre in Katowice: average daily cloud cover, temperature, relative humidity, precipitation, and insolation. Data were pertinent to the dates of blood and urine sampling. Meteorological conditions were similar on all days when biological samples were obtained.

The Bioethical Committee of the Silesian Medical University of Katowice approved the protocol of the study.

Data were stored in the dedicated database prepared in MS EXCEL. Statistical analysis was performed with Statgraphics v. 2.6. Statistical significance for qualitative variables in contingency tables was assessed with the Pearson chi-square test. Difference among groups was evaluated with univariate analysis of variance (ANOVA). The NIR test was used to find the least significant difference. A p-value of 0.05 was considered statistically significant.

Results

The group of 15 patients with early RRMS (age range: 19-51 years) comprised 10 women (mean age 32.7 years) and 5 men (mean age 35.0 years). The EDSS score ranged from 0.5 to 2.0. The group of 15 patients with advanced RRMS (age range: 30-58 years) com-

Table 1. Calcium, phosphorus, and related parameters among studied patients and controls*

	Patients with early RRMS	Patients with advanced RRMS	Controls
Serum phosphorus [mmol/L]	1.13 ± 0.20 (0.59-1.41)	1.01 ± 0.17 (0.54-1.23)	1.03 ± 0.14 (0.71-1.27)
Serum calcium [mmol/L]	2.37 ± 0.20 (1.99-2.98)	2.39 ± 0.06 (2.24-2.47)	2.32 ± 0.06 (2.25-2.42)
Alkaline phosphatase (ALP2L) [U/L]	63.87 ± 16.84 (37-94)	63.53 ± 18.26 (39-98)	60.67 ± 24.21 (38-119)
Bone alkaline phosphatase (ALP) [U/L]	22.80 ± 7.70 (10-35)	22.67 ± 7.03 (14-38)	20.40 ± 10.62 (8-50)
Ionized calcium [mmol/L]	1.14 ± 0.06 (1.07-1.31)	1.09 ± 0.05 (0.95-1.17)	1.17 ± 0.19 (1.02-1.82)
Parathormone [pg/mL]	61.01 ± 34.97 (5.76-140)	78.78 ± 40.36 (30.49-189.7)	55.15 ± 26.71 (22.68-120.6)
$25(OH)D_3$ [ng/mL]	16.91 ± 8.44 (6.61-31.94)	13.59 ± 6.54 (4-26.77)	25.28 ± 8.79 (11.23-40)
Calcium in 24-hour urine collection [mmol/L/24 h]	2.67 ± 1.90 (1.16-7.57)	3.14 ± 2.52 (1.00-8.22)	4.29 ± 2.87 (0.86-10.19)

RRMS – relapsing-remitting multiple sclerosis
**Values reported as mean ± standard deviation (range)*

prised 11 women (mean age 36.8 years) and 4 men (mean age 38.2 years). The EDSS score ranged from 1.0 to 3.0.

In 60% of patients with early RRMS and in 66.67% of patients with advanced RRMS, the disease was diagnosed in the winter-spring period with the weather conditions typical for those seasons.

Results of patients with RRMS were compared with the control group (age range: 26-60 years) which consisted of 10 women (mean age 35.2 years) and 5 men (mean age 39.0 years) who had neither focal damage of the CNS nor metabolic disorders due to other conditions.

The study was performed in similar weather conditions. All patients and controls were white-collar workers.

Neither MS patients in either stage of the disease, nor controls had any abnormalities of calcium and phosphorus metabolism. The findings were within the reference ranges (Table 1).

The only significant difference shown in the analysis of variance among studied groups was related to the 25(OH)D_3 concentrations. The test did not reveal any significant relationship for other cases (Table 2). A post-hoc NIR test showed significant differences in mean concentrations of 25(OH)D_3 [ng/mL] between (1) patients with early MS and controls (controls had higher mean values) ($p < 0.006$); (2) patients with advanced MS and controls (controls had higher mean values) ($p < 0.0002$). Patients with early and advanced MS did not differ regarding 25(OH)D_3 concentrations ($p = 0.2617$).

Among patients with advanced MS, women had significantly lower 25(OH)D_3 concentrations than men. In patients with early MS, men had higher 25(OH)D_3 concentrations but the difference was less robust.

In MS patients, regardless of the stage, the mean 25(OH)D_3 serum concentration was lower than in controls in the same weather conditions. Patients with early MS and with a lower number of relapses had significantly higher 25(OH)D_3 concentrations.

Dietary supplements containing 25(OH)D_3 were used more often by the patients with early RRMS (10/15, 66.7%) than among patients with advanced RRMS (6/15, 40%) or controls (0/15) ($p < 0.00063$ for the difference between patients with MS and controls). Similarly, dietary supplements containing unsaturated fatty acids were used more often by the patients with early RRMS (7/15, 46.7%) than among patients with advanced RRMS (5/15, 33.3%) or controls (1/15, 6.7%) ($p < 0.048$ for the difference between patients with MS and controls).

Table 2. Analysis of variance for the parameters of calcium-phosphate metabolism among studied groups

	F-test	*P*-value
Serum phosphorus [mmol/L]	2.140	0.130
Serum calcium [mmol/L]	1.301	0.283
Alkaline phosphatase (ALP2L) [U/L]	0.116	0.891
Bone alkaline phosphatase (ALP) [U/L]	0.370	0.693
Ionized calcium [mmol/L]	1.939	0.156
Parathormone [pg/mL]	1.911	0.161
25(OH)D_3 [ng/mL]	8.544	> 0.001
Calcium in 24-hour urine collection [mmol/L/24 h]	1.725	0.191

MS patients, especially those in an early stage of the disease, significantly more often than controls use multivitamin preparations (120 IU of 25(OH)D_3, on average) and polyunsaturated fatty acids (625 mg daily, on average). Among patients with early MS, the beginning of the oral supplementation was associated with the time of diagnosis, while in patients with advanced MS the beginning of supplementation took place 3-4 years on average after the diagnosis, i.e. two years before this study was started.

Discussion

As early as in 1974, Goldberg hypothesized that 25(OH)D_3 deficiency related to lower exposure to sunlight might be an environmental factor that increases the risk of MS [14].

It is highlighted that the incidence of MS among Caucasians is higher in areas of lower insolation [7,8, 15]. The beneficial impact of sunlight is explained with the vitamin D synthesis under the influence of ultraviolet radiation, as vitamin D has some immunomodulating properties [8-10].

According to Napiórkowska and Kuchuk, the most significant deficiency of 25(OH)D_3 in women is noted at the age of the greatest fertility, i.e. between 15 and 45, which coincides with the peak incidence of MS [16,17]. It is estimated that about 4-40% of women at that age suffer from vitamin D deficiency.

Our study showed significantly lower 25(OH)D_3 serum concentrations among MS patients, regardless of the stage of their disease, than in controls. In patients with the disease duration of 5-6 years, the 25(OH)D_3

concentration was lower. Additionally, among patients with advanced MS, markedly lower 25(OH)D_3 concentrations were noted in women than in men with the same level of motor disability [17].

This study also assessed the mean 25(OH)D_3 serum concentration in relation to the number of relapses. Concentrations of 25(OH)D_3 were significantly higher among patients with early MS and with a lower number of relapses.

Significantly more common use of dietary supplements, including those with 25(OH)D_3, does not affect the 25(OH)D_3 concentrations in both groups of MS patients, which are lower than in controls.

MS patients, especially those in an early stage of the disease, use 25(OH)D_3 supplementation more commonly than controls. The same difference is seen regarding the use of polyunsaturated fatty acids. A special role is ascribed to linolenic acid because of its beneficial effect shown in several clinical trials [18] and probably this information results in great popularity of that agent among MS patients.

Conclusions

1. MS patients have lower 25(OH)D_3 serum concentrations than controls; it is more marked among women and in patients with longer disease duration.
2. Lower 25(OH)D_3 concentrations may be modified with the significantly more common use of multivitamin preparations and polyunsaturated fatty acids by MS patients.

Disclosure

Authors report no conflict of interest.

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