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Bone metabolism and vitamin D status in patients with multiple sclerosis

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ABSTRACT

Background: Vitamin D (VD), an important factor for bone health immobilization and immune regulation, has been shown to have low serum concentration in multiple sclerosis (MS) patients. Those patients have also multiple fracture risk factors, including progressive immobilization and long-term glucocorticoids treatment. The aim of the study was to analyze bone health (osteopenia or osteoporosis prevalence) and VD serum concentration in MS patients as well as the influence of disease activity and treatment on bone health.

Materials and methods: The study involved 72 MS patients: 52 women and 20 men. Mean age was 40.3 ± 10.5 yrs, mean EDSS (Expanded Disability Status Scale) 3.3 ± 1.9. Bone health was analyzed using standard densitometry in the lumbar spine and femoral neck. Serum levels of VD, calcium, phosphate and parathormone were assessed. We compared two groups of patients with multiple sclerosis: relapsing - remitting MS (RRMS) and progressive relapsing MS (PRMS).

Results: Densitometry revealed osteopenia in twenty-six (36.1%) patients and osteoporosis in eleven (15.3%), no bone fractures were presented. Sixty-eight MS patients (94.4%) had lower VD serum level if compared to population referential values. Thirteen patients (18.1%) had severe VD deficiency. Densitometry parameter (T-score of the lumbar spine) worsened with EDSS increase ($r = -0.43$, $P = 0.001$). There was a statistically significant negative correlation between VD concentration and EDSS score ($r = -0.31$; $P = 0.009$).

Conclusions: Our study indicates that patients with MS have high incidence of osteopenia and osteoporosis and vitamin D deficiency. Bone health disturbances studied by densitometry are related to the disability caused by MS.

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1. Introduction

Multiple sclerosis (MS) is a chronic progressive, inflammatory and demyelinating disease of the central nervous system. Disease onset usually occurs in early adulthood, and MS is more common in women. Patients can suffer from various neurological symptoms and signs, including visual and pyramidal signs, cranial nerve disturbances, cerebellar, sensory and bowel or bladder dysfunction, as well as cognitive impairment. Symptoms may worsen with disease progression, leading to disability and immobilization.

It is suspected that environmental factors, such as sunlight exposure, play a crucial role in the disease etiopathogenesis. The results of several studies suggest that low exposure to sunlight correlates with higher MS prevalence [1,2] and that the role of UVB light in vitamin D synthesis is critical for this phenomenon [3].

Although vitamin D and its metabolites are widely known to be vital for calcium homeostasis [4], they also play a very important role in the modulation of the immune response. Low vitamin D concentration correlates with higher MS prevalence [5]. Vitamin D promotes an anti-inflammatory response by increasing the activity of regulatory lymphocytes. It also inhibits the proliferation of CD4+ T cells and MBP specific T cells. Active vitamin D metabolites reduce the number of IL-6- and IL-17-secreting cells and increase the number of Il-10-secreting cells. The presence of active vitamin D metabolites increases the number of vitamin D receptors on both inhibited and activated cells and enhances 2,3-deoxygenase expression. This enzyme is crucial for the increase in regulatory cell number (CD4+ and CD25+) [6,7].

It is unknown that why patients with MS have lower vitamin D concentrations [8]. It might be caused by a combination of low dietary vitamin D intake and reduced sunlight exposure. MS symptoms may worsen due to heat, leading patients to avoid the sun. Reduced sun exposure might also be caused by increasing disability and immobilization during MS progression. There is a direct link between sunlight exposure and vitamin D synthesis in the skin.

Osteoporosis is a progressive bone disease that is characterized by a decrease in bone mass and density, which can lead to an increased risk of bone fractures. Dual-energy X-ray absorptiometry (DXA) plays an important role in the diagnosis of osteoporosis and increased fracture risk. The densitometry diagnostic criterion of osteoporosis is the reduction of bone density of the proximal femur or lumbar spine expressed as a T-score ≤ -2.5.

There is also strong evidence supporting a link between T cell activity and bone loss during estrogen deficiency. These data are consistent with the interactions of vitamin D and the immune system and suggest that a low concentration of vitamin D might act as a proinflammatory factor [9].

In MS, osteoporosis risk may be related to impaired mobility, lack of physical activity, and low VD levels. All of these factors, especially progressive immobilization, can lead directly to bone health disturbances [10–12].

The aims of this project were to study bone health in MS patients using densitometry, clinical and biochemical parameters, and vitamin D serum concentration and to find an association between disease activity and bone health. We compared two groups of MS patients: relapsing-remitting MS (RR MS) and progressive-relapsing MS (PR MS).

2. Patients and methods

The Bioethical Committee approved this study. Participants provided written consent to participate in this study. Participants received a questionnaire that included information about the study, read the information and, after speaking with a doctor, agreed to participate. Both, blood tests and DXA were performed during their hospitalization.

Seventy-two patients (52 females and 20 males; mean age 40.3 ± 10.5 yrs (ranges: 21–58 yrs)) with a confirmed diagnosis of MS, according to the McDonald and Polman criteria [13], were included in the study. All patients were recruited from the Department of Neurology. The mean EDSS (Expanded Disability Status Scale) score was 3.3 ± 1.9 (ranges: 1.0–8.5). Forty-two patients suffered from relapsing-remitting MS (RR MS). Thirty patients had progressive MS: twenty-two patients had progressive-relapsing MS (PR MS) and eight had secondary-progressive MS (SP MS). Finally in this study, we compared two groups of patients: RR MS and PR MS. The SP MS group was not included because of the small number of patients. The medical history of all patients included intravenous administration of methylprednisolone (5 g/treatment) at least once in the year prior to the study. Fifty-eight patients received long-term disease modifying therapy including beta interferons (IFN-β), glatiramer acetate, or immunosuppressive treatment. The majority of the patients were treated with immunosuppressive drugs (32 with mitoxantrone and 5 with cyclophosphamide). The rest of the patients ($n = 24$) were treated with immunomodulating drugs; 16 received IFN-β, 8 received glatiramer acetate, and one received natalizumab. Ten patients remained without chronic immunotherapy.

Bone health was assessed with DXA (Discovery A, Hologic) in the lumbar spine (L1-L4 in the AP projection) and the proximal femur (femoral neck) and was presented as a T-score. T-score values of -2.5 or lower were defined as osteoporosis, and T-score values lower than -1.0 and higher than -2.5 were defined as osteopenia. Additionally we evaluated the incidence of low-energy bone fractures studying the patients' medical histories.

Laboratory tests included serum concentrations of vitamin D, calcium, and intact parathyroid hormone (intact-PTH) levels. 25 hydroxyvitamin D3 isoform was measured. 25-hydroxyvitamin D concentration was assessed by a chemiluminescence immunoassay with standard kits Liaison. Other biochemical parameters in blood were assessed using routine laboratory methods.

There was no control group. In our analysis, the vitamin D concentrations in MS patients were compared to a reference group [14]. Population referential values for vitamin D concentration in the serum (matched for age) ranged from 30 to 80 ng/ml. A slight vitamin D deficiency ranged from 20 to 30 ng/ml, a moderate vitamin D deficiency was 10–20 ng/ml, and a severe VD deficiency ranged from 0 to 10 ng/ml [14]. Our assessment of vitamin D level was performed in winter (December and January) and in summer (July, August). We

excluded the significant differences after adjustment for seasonal variation. In our project we based on recommendations for Central Europe. These guidelines do not take into the consideration a seasonal variability of VD concentrations [15].

To exclude other pathologies that could affect bone health, thyroid hormone status, renal parameters, BMI (*body mass index*) and life style choices such as smoking were analyzed. Patients were not included in the study if those parameters were abnormal.

Statistical analyses were performed using the SAS System version 9.3. Data are presented as the mean ± standard deviation. Associations between quantitative variables were investigated using Pearson's rank correlation test. Statistical significance was established as $P < 0.05$. A comparison of MS patients groups with relapsing-remitting MS (RR MS) and progressive-relapsing MS (PR MS), in terms of quantitative parameters, was performed using the Mann–Whitney test.

3. Results

Densitometry revealed bone health abnormalities in 37 patients (51.4%): osteopenia in twenty-six patients (36.1%), and osteoporosis in eleven patients (15.3%). Densitometry of the proximal femur showed osteoporosis in 9 patients (12.5%) and osteopenia in 20 patients (27.8%). Densitometry of the lumbar spine revealed osteoporosis in 7 patients (9.7%), and 24 patients (33.3%) had osteopenia. According to patients' medical history, no low-energy bone fractures had occurred in the study group.

Sixty-eight (94.4%) patients had low serum vitamin D levels (<30 ng/ml), and in 13 of those patients (18.1%), the deficit was severe (<10 ng/ml). The means vitamin D concentration in MS patients was 18.8 ng/ml and was significantly lower than referential values. The mean vitamin D concentration was 12.84 ng/ml in winter and 21.93 ng/ml in summer. Patients with osteoporosis confirmed by densitometry had a lower vitamin D concentration (mean: 13.1 ng/ml) than patients with osteopenia (mean: 21.9 ng/ml). No significant abnormalities were found in calcium and phosphate serum concentrations. Ten patients (13.9%) had abnormal PTH concentrations; six of those patients had high PTH levels (>65 pg/ml), and 4 had low PTH levels (<15 pg/ml). The mean BMI was 23.6 kg/m^2. BMI did not correlate with BMD. Among the 72 patients no one smoked.

Densitometry parameters (*T*-scores of the lumbar spine but not the femoral neck) worsened as EDSS increased ($r = -0.43$; $P = 0.001$) (Fig. 1). There was a statistically significant negative correlation between VD concentration and EDSS score ($r = -0.31$; $P = 0.009$) (Fig. 2). We did not find any correlation between vitamin D serum concentration and disease duration ($P = 0.46$) and densitometry parameters ($P = 0.34$). There was no correlation between serum PTH concentration, densitometry parameters, and EDSS scores.

According to our results, the median vitamin D concentration was significantly lower in PR MS patients than in RR MS patients (10.3 ng/ml vs. 15.8 ng/ml, $P = 0.004$). This result might be explained by significant age differences; patients who suffered from PR MS were older than those who suffered from RR MS ($P = 0.0068$). Moreover, there was a significant difference in the degree of disability. Patients with PR MS scored

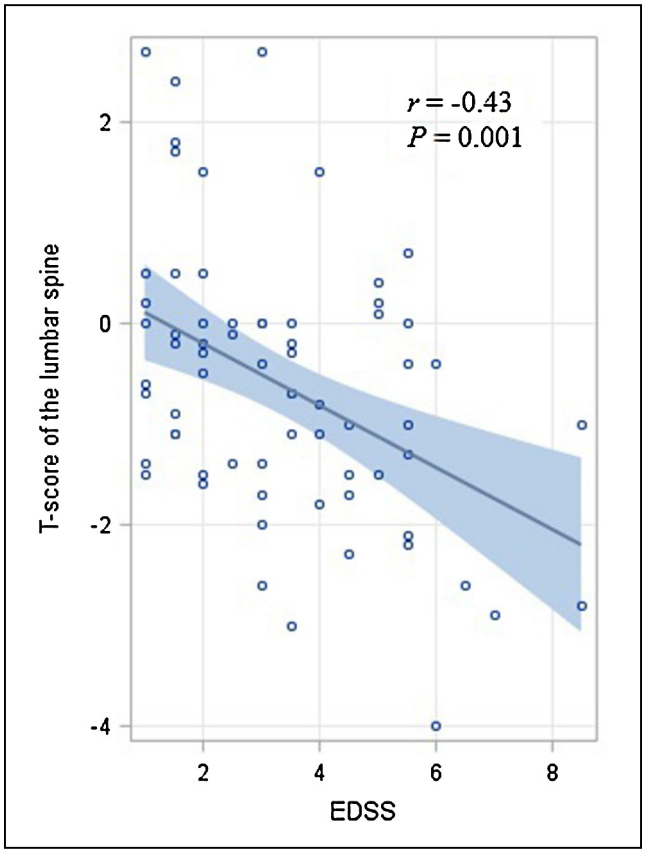

Fig. 1 – The correlation between the densitometry results: *T*-scores of the lumbar spine and EDSS scores in patients with multiple sclerosis ($r = -0.43$; $P = 0.001$).

significantly higher in the EDSS; the mean EDSS score was in this group 5.0, while in RR MS patients it was significantly lower (mean EDSS = 2.0).

When we compared these two groups of MS patients separately, we did not find any significant correlation between the densitometry parameter (*T*-score of the lumbar spine) and the EDSS score ($P = 0.310$ for PR MS patients; $P = 0.072$ for RR MS patients). Vitamin D concentration did not correlate with EDSS score in either PR MS patients ($P = 0.380$) nor in RR MS patients ($P = 0.21$).

Patients in the PR MS group with longer disease duration have a significantly increased risk of osteoporosis; the median *T*-score of the proximal femur was in this group was −1.1 compared to RR MS patients, where it was −0.6 ($P = 0.0426$). Demographic data of MS patients are presented in Table 1. Laboratory and clinical data are shown in Table 2.

4. Discussion

According to our results, the bone health studied with DXA has revealed abnormalities in MS patients. However it is difficult to conclude whether the BMD loss is lower in MS patients than in the general population. Moen et al. have found high prevalence of low bone mass in patients with MS and in the general population as well. They measured BMD at early stages of

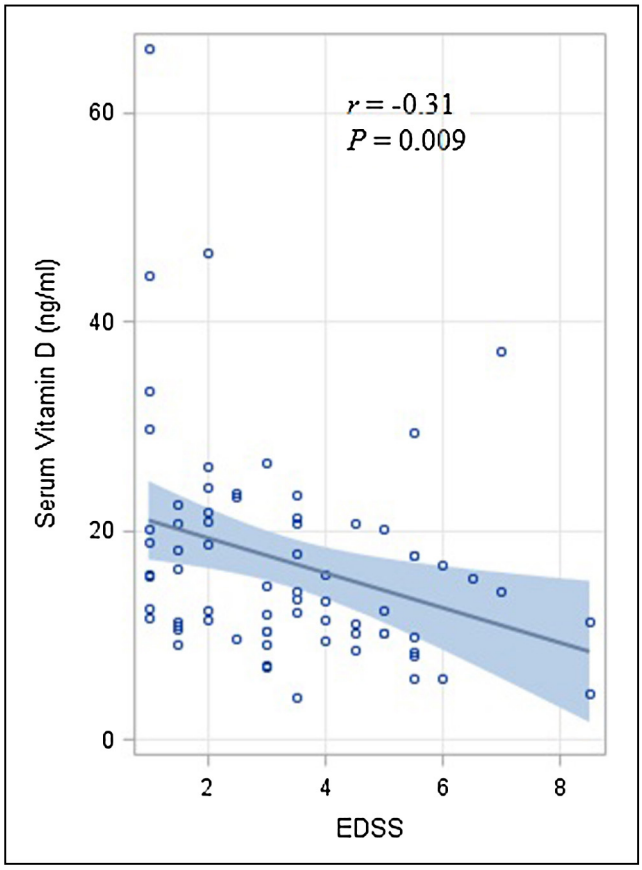

Fig. 2 – The correlation between serum concentrations of vitamin D (ng/ml) and EDSS scores in patients with multiple sclerosis ($r = -0.31$; $P = 0.009$).

disease. It is well known that osteoporosis is common in patients with multiple sclerosis with long-standing disease. But in this study, low bone mass was appeared early in MS. This finding is compatible with the hypothesis that MS and osteoporosis share etiological factors, and the bone deficit in the newly diagnosed patients could be explained by low bone mass before disease onset [16].

In our study, densitometry revealed that 36.1% of patients had osteopenia. The prevalence of osteoporosis reached 15.3%. This finding could be attributed to disability and the prolonged immobilization of patients, as both are well known risk factors for bone mass loss. Densitometry parameters (*T*-score of the lumbar spine but not of the proximal femur) worsened as EDSS increased. Our results are in line with those from other studies. Khachanova et al. reported that reduced bone mineral density in the lumbar spine and femoral neck is associated with a higher EDSS score and is caused by the combination of moderate pyramidal and cerebellar dysfunction [17].

Immobility as a major factor of BMD loss was suggested by Ayatollahi et al. Their results showed that BMD was significantly lower in MS patients with higher EDSS score and longer disease duration. Moreover, femoral BMD was significantly lower among MS patients than age matched controls [18].

Some literature data suggest that MS patients have higher risk for osteoporosis. Coskun et al. determined risk factors of low BMD in patients with MS. Of the 67 patients, 20.9% revealed low BMD on femoral neck densitometry. Longer disease duration with severe disability led to lower BMD [19].

To give an impression that the prevalence of bone deficiency is actually higher in MS patients we cite the meta-analysis of Huang et al. This analysis shows that MS

Table 1 – MS patients. Demographic data.

Variable	Progressive-relapsing MS ($n = 22$) F: 10; M: 10		Relapsing-remitting MS ($n = 42$) F: 29; M: 13		P-value
	Median	Q1–Q3	Median	Q1–Q3	
Age (years)	45	36–53	36	28–47	0.0068*
EDSS	5	3.5–5.5	2	1.3–3.0	0.0000*
MS duration (years)	9	8–12	5.5	4–9	0.0003*

EDSS – Expanded Disability Status Scale; F – females; M – males; MS – multiple sclerosis; Q1, Q3 – quartiles.
* Statistically significant difference.

Table 2 – MS patients. Laboratory data.

Variable	Progressive-relapsing MS ($n = 22$) F: 10; M: 10		Relapsing-remitting MS ($n = 42$) F: 29; M: 13		P-value
	Median	Q1–Q3	Median	Q1–Q3	
DXA (*T*-score of the lumbar spine)	−1	−1.7 to 0	−0.3	−1.1 to 0.2	0.1376
DXA (*T*-score of the proximal femur)	−1.1	−1.9 to −0.4	−0.6	−1.3 to −0.3	0.0426*
Serum vitamin D (ng/ml)	10.3	8.34–16.7	15.8	11.5–21.31	0.0049*
Serum calcium (mg/dl)	2.33	2.29–2.38	2.345	2.24–2.38	0.7824
Serum PTH (mg/dl)	42.74	31.9–52.8	32.93	27.87–42.54	0.0491*

DXA – densitometry; F – females; M – males; MS – multiple sclerosis; PTH – parathyroid hormone; Q1, Q3 – quartiles.
* Statistically significant.

patients are at high risk of osteoporosis if compared with healthy controls. Furthermore disease duration over 7 years, glucocorticosteroids administration as well as EDSS over 3 are risk factors for reduced BMD [20].

Obtained results suggest that there is a strong evidence that MS is associated with an increased risk of osteoporosis. It can be related to the cumulative effects of various factors, such as physical inactivity and reduced mechanical load on the bones (offsetting gravity). Another possible risk factor of osteoporosis is low vitamin D levels. The role of the inflammatory processes related to the underlying disease is considered in the context of complex bone metabolism [21].

Most epidemiological studies suggest that vitamin D deficiency is frequent in central European populations [14]. Seasonal variability of vitamin D serum concentrations is reported by many authors although obtained results are heterogeneous and still discussed. We based on recommendations (published in 2013) for serum vitamin D concentrations. The authors of these guidelines did not taken into the considerations the data related to the seasonal variability [15].

In the literature, vitamin D deficiency has been reported in approximately 70% of seriously disabled patients with MS. The results of a meta-analysis confirm also that MS patients had decreased mean levels of vitamin D [22].

In some paper we may found also an opinion that vitamin D status did not differ significantly between MS patients and controls [23].

On the other hand, there are some studies which confirm our results. Thirty-one patients with MS and thirty matched healthy controls were participated in this study. MS patients had significantly lower vitamin D levels (17.3 ng/ml vs 43.1 ng/ml; $P < 0.001$) compared to controls. Moreover, those patients had also significantly lower BMD at the lumbar spine and femur trochanter densitometry if compared to the matched controls [24].

In another one, levels of vitamin D were also lower in MS patients than controls. Such a result was explained by differences in climate and geography. In contrast to our results, there was no association between vitamin D status and disease severity [25].

Harandi et al. reported that vitamin D could be involved in the regulation of clinical disease activity in MS patients, based on its inverse correlation with disease severity measured using EDSS scores [26]. Shahbeigi et al. also found a significant correlation between lower VD status and higher EDSS [27]. Our study confirms the possible importance of vitamin D status in patients with MS and its association with disease severity. Patients with higher scores on EDSS had lower levels of serum vitamin D ($P = 0.009$). It is, however, difficult to explain whether VD deficiency is a pathogenic factor for MS or if vitamin D simply decreases with the patient's increasing disability. However, obtained results seem to confirm the influence of disease activity on bone health.

In the literature we have found also a study performed on polish population. This report confirmed significantly lower vitamin D concentrations among MS patients compared to controls. Moreover, in patients with longer disease duration (5–6 years) this concentration was lower than patients at the early stage of MS. These results confirm our observation. Patients in the PR MS group with longer disease durations had significantly lower vitamin D concentration. Patients with early MS had higher vitamin D levels [28].

It is important to point out that use of dietary supplements including vitamin D did not affect the vitamin D concentrations in both groups of MS patients. Moreover, vitamin D supplementation did not prevent bone loss over a 2-year observation period in a placebo controlled trial. Percentage change in BMD did not differ between participants who had received vitamin D and who had received placebo [29].

Vitamin D plays a role in the pathogenesis and prevention of several diseases other than MS, such as cancer, cardiovascular diseases, diabetes, rheumatoid arthritis, and other autoimmune diseases. Meta-analyses and systematic reviews have revealed an association between vitamin D insufficiency or deficiency and diseases that do not affect directly the bone health [30].

In this study, we noted decreased vitamin D levels. Patients with osteoporosis had lower VD concentrations than patients with osteopenia (the mean concentration of vitamin D in the first group was 13.1 ng/ml vs. 21.9 ng/ml in MS patients with osteopenia). A more pronounced vitamin D insufficiency was observed in patients with RP MS. In this group of patients, low VD values can be associated with low sunlight exposure that was caused by increasing disability and immobilization during related to the disease progression. It might also be explained by significant age differences; patients who suffered from PR MS were older than those with RR MS ($P = 0.0068$). Analyzing *T*-score or vitamin D concentration with EDSS in those groups we did not find any significant correlation. This fact could be explained by the low number of those patients in our material.

Glucocorticoids are frequently used to treat MS relapses. Glucocorticoid-induced osteoporosis is the main type of secondary osteoporosis and leads to fractures far more often than postmenopausal osteoporosis. Epidemiological studies have shown that fracture risk has increased rapidly after the onset of oral glucocorticoid treatment and was related to the dose and duration of glucocorticoid exposure [31]. Prolonged oral corticosteroid treatment using more than 5 mg of prednisolone (or equivalent) daily leads to a reduction in bone mineral density and a rapid increase of fracture risk during the treatment period [32].

Glucocorticoids are widely used for the chronic treatment of autoimmune inflammatory diseases, such as rheumatoid arthritis. Overall, current evidence supports the thesis that bone loss is a disease independently related to both rheumatoid arthritis and glucocorticoid use [33].

In our study, all tested patients received only short-lasting, high-dose-methylprednisolone therapy at least once in the year prior to the study. Intravenous infusions of high-dose methylprednisolone in MS patients did not result in a severe decrease in densitometric parameters.

Another study revealed that MS patients who have received short-term high-dose glucocorticoids treatment are not at increased risk of low bone mass. BMD was compared to a healthy age-matched reference population [34]. The findings cohere with ours. Glucocorticosteroids treatment did not seem to be a cause of secondary osteoporosis in MS patients.

Zorzon et al. [35] support this observation. Osteopenia was found only in patients treated for relapses (they received steroids for a short time), and they had a significantly

increased EDSS. It seems that bone health in MS patients depends more on disease activity than on glucocorticosteroid administration.

In Poland, there are no precise epidemiological data about prevalence of osteoporosis/osteopenia in the general population. We found only the information on the incidence of hip fractures in Poland. This study was however conducted on a large number of patients. The results of this study confirmed the high incidence of hip fractures in Poland [36].

It is shown in the literature that bone fractures can occur more often in patients with MS. According to Bazelier et al., patients with MS have an increased risk of osteoporotic fractures, especially hip fractures. The risk was higher in patients who had recently used oral or intravenous glucocorticosteroids or antidepressants [37,38]. In our study, no patients had low-energy bone fractures in their medical histories, perhaps because our study group consisted on relatively young patients (mean age was 40.3 ± 10.5 yrs).

5. Conclusions

According to our results, patients with multiple sclerosis have a high incidence of osteopenia, osteoporosis and vitamin D deficiency. Osteopenia occurs more often than osteoporosis. Densitometry parameters (*T*-score of the lumbar spine) worsened as EDSS increased. Vitamin D negatively correlated with EDSS score.

Conflict of interest

None declared.

Acknowledgement and financial support

None declared.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

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