Original Article

A PROSPECTIVE STUDY ON 25 HYDROXYVITAMIN D SERUM LEVELS IN PATIENTS WITH RELAPSING-REMITTING MULTIPLE SCLEROSIS

Georgi S. Slavov,
Maria G. Manova,
Anastasiya G. Trenova,
Ivanka I. Kostadinova[1],
Pavel I. Pavlov[2],
Nonka G. Mateva[3],
Zahari I. Zahariev

Department of Neurology,
University Hospital "St. George",
Medical University – Plovdiv
[1]Department of Pharmacology and Clinical Pharmagology,
Medical University – Plovdiv
[2]Department of Central laboratory,
St. George University Hospital,
Medical University – Plovdiv
[3]Department of Public health,
Medical University – Plovdiv

Corresponding Author:
Georgi S. Slavov
e-mail: georgi.slavov.15130@gmail.com

Received: October 27, 2014
Revision received: November 21, 2014
Accepted: November 24, 2014

Summary

To assess the changes in the serum concentrations of 25(OH)D in relapse and remission and their relation to the degree of neurological deficit. We analyzed 53 subjects (30 controls and 23 patients) from October 2012 to May 2013. Diagnosis was based on McDonald 2010 criteria. The severity of neurological deficit was assessed by the Expanded Disability Status Scale. Serum concentrations of 25(OH)D (nmol/l) were measured by ELISA once in controls and twice in patients – in relapse and remission. Mean levels in all groups were in the mild to moderate deficiency range, being lowest in the patients in relapse (controls 31.46±7.3; relapse 26.93±7.44; remission 28.06±7.28). There was a trend for lower levels of 25(OH)D in healthy females in comparison to their male counterparts, and in female patients in relapse as compared to males (female controls - 26.56±8.4, male controls - 41.35±11.86; females in relapse - 26.33±10.03, males in relapse – 28.06±11.08). Negative statistically significant correlations between the concentrations of 25(OH)D during relapse and remission and the degree of neurological deficit in the corresponding period were found (cc. -0.593, Sig 0.03, relapse; -0.46, Sig 0.024, remission). Assessment of the risk for development of MS, regarding the 25(OH)D showed protective effect with respect to the risk of disease occurrence (OR: 04125 relapse; 0.578 remission).
Keywords: vitamin D, multiple sclerosis, EDSS

Introduction

Multiple sclerosis (MS) is an immune organ-specific disease with unknown aetiology and treatment of limited efficacy. Epidemiological studies determine a northern-southern gradient in the distribution of MS in the world. Further from the equator the morbidity increases up to 100/100000 people in some northern regions [1]. Twin- and population-genetic studies support the hypothesis for a moderate hereditary risk, modified to a different extent by exogenous factors [2, 3]. Vitamin D, a secosterol hormone and an environmental factor, if applied before the induction with myelin protein, prevents the development of experimental and allergic encephalomyelitis (EAE) and retains the progressive

course when applied after the first clinical signs have occurred [4, 5]. Between 70 and 80% of Vitamin D in the organism is synthesized as cholecalciferol (D3) in the skin following ultraviolet radiation, and 10% are taken in with the food (ergocalciferol, D2) [6, 7]. The concentration of 25 hydroxyvitamin D (25(OH)D is an indicator for the status of vitamin D. A multicentre investigation in Bulgaria in 2012 defined the following ranges applicable to the Bulgarian population, regarding 25 hydroxyvitamin D levels: sufficient ⩾ 50 nmol/l, mild to moderate deficiency – 25-49.99 nmol/l, and severe deficiency ⩽ 25 nmol/l. These levels are identical with the ones indicated by the consensus conference in Germany in 2012, which allows comparative analysis of Bulgarian data and data of foreign authors [6]. Clinical observations determine a relation between the changes in 25(OH)D concentrations and the risk of developing MS, as well as non-unidirectional results, regarding their correlation with the severity of the neurological deficit, coefficient of exacerbations, Gd-enhanced lesions, oligoclonal cerebro-spinal fluid stripes [8,9,10]. In our country the morbidity of MS has risen twice for a period of 16 years (1983-1999) [11, 12]. From this point of view, studies on the role of vitamin D in the aetiology and pathogenesis of MS are justified and necessary.

To assess the changes in the serum concentrations of 25(OH)D in relapse and remission periods and their relation to the degree of the neurological deficit.

Materials and Methods

This is a prospective, case-control study. Outpatients were selected from the centre for diagnosis and treatment of MS at St. George University Hospital in Plovdiv during the astronomical winter period between October 2012 and May 2013. The study was approved by the Ethics Committee at the Medical University – Plovdiv with protocol N-3/05.07.2012 and was funded by Medical University-Plovdiv as a university research project. All subjects that participated in the study signed an informed consent prior to inclusion. According to the study design, 25(OH)D serum concentrations were tested once in controls and twice in patients – in relapse and remission periods, respectively (⩾ 2 months after relapse). Patients with relapse were admitted to the Neurology Clinic at St. George University Hospital in Plovdiv and were treated with Methylprednisolone 500 mg i.v. in the mornings up to a cumulative dose of 2500 mg.

Subjects

Data from 53 subjects were analyzed (30 controls - 15 males, 15 females) and 23 patients (15 females, 8 males). The patients were followed up during the time of relapse and on an outpatient basis during remission.

Patients

Inclusion criteria: Caucasians living in the area between 41^0-42^0 northern latitude in the age span of 15-50 years, with relapsing-remitting MS and EDSS severity of the neurological deficit 1.5-5.0.

Exclusion criteria: primary and secondary progressive course of disease; treatment with medications, modifying the disease course in the year prior to registration; vitamin D treatment and medications, influencing vitamin D metabolism (oral contraceptives, antiepileptic drugs, laxatives, thiazide diuretics), concomitant autoimmune disorders, acute and chronic infections, liver, kidney, neoplastic diseases, hyperparathyroidism, diabetes mellitus, hypercalcemia.

Controls

Inclusion criteria: clinically healthy subjects, not using oral contraceptive drugs, hormone-replacement therapy, laxatives, polyvitamins.

Clinical methods

Diagnosis was based on 2010 McDonald criteria. The severity of the neurological deficit was assessed by the Expanded Disability Status Scale (EDSS, Kurtzke, 1983). A relapse was defined as occurrence of new neurological signs or impairment of old ones for >24 hours, increase of EDSS score ⩾ 0.5 without fever and following a period of at least 30-days improvement or stable condition after a relapse.

Laboratory methods

Venous blood samples were collected in the morning. After centrifugation, the separated sera were frozen and stored at -20°C until analysis. Serum concentrations of vitamin D were determined by enzyme-linked immunosorbent assay with original ELISA-kits, Immundiagnostik AG, Germany. Each sample was tested by means of *duplicate analyses and m*easured serially by ELISA-reader (Sirio-microplate reader, SEAC-Italy) at 450 nm and 620 nm.

Statistical methods

The following statistical tests were used: Kolmogorov-Smyrnov test to determine distribution; Indipendent Samples T-test where normal distribution is present; Spearman's correlation; Mann-Whitney U – test in case of non-uniform distribution; Odds Ratio.

Results

The clinical characteristics of the patients are presented on Table 1.

Table 1. Clinical characteristics of investigated patients

Parameter	*n*	*Gender*		Age mean ± SEM	Disease duration mean ± SEM	Age at disease onset mean ± SEM
Group		female	male			
		n	n			
Controls	30	15	15	31.17±1.41		
Patients	23	15	8	35.09±2.87	7.65±1.75	27.30±1.97

No statistically significant difference was found between patients and controls, as well as between males and females, regarding age. All patients were registered in the first month of a subsequent relapse (between the 3rd and the 25th day), and the biggest share of cases were registered before the end of the 3rd week (47.83%, n=11). The average degree of neurological deficit in relapse was 2.48±0.19, during remission – 1.72±0.19. The parameter decreased statistically significantly during remission, as compared to the relapse period ($p<0.001$).

No statistically significant differences were present between controls, patients in relapse and patients in remission in the serum concentrations of 25(OH)D. However, certain trends were marked and are worth mentioning. Mean levels in all groups (both controls and patients) were in the mild to moderate deficiency range, being lowest in the patients in relapse (healthy controls-31.46±7.3 nmol/L^{-1}; patients in relapse-26.93±7.44 nmol/L^{-1}; patients in remission-28.06±7.28 nmol/L^{-1}). There was a trend for lower levels of 25(OH)D in healthy females in comparison to their male counterparts, and in female patients in relapse as compared to males in relapse (female controls – 26. 56±8.14 nmol/L^{-1}, male controls – 41.35±1.86 nmol/L^{-1}; females in relapse-26.33±10.03 nmol/L^{-1}, males in relapse-28.06±11.08 nmol/L^{-1}). During remission its levels rose but not to a point of statistical significance.

The results are presented on Fig 1.

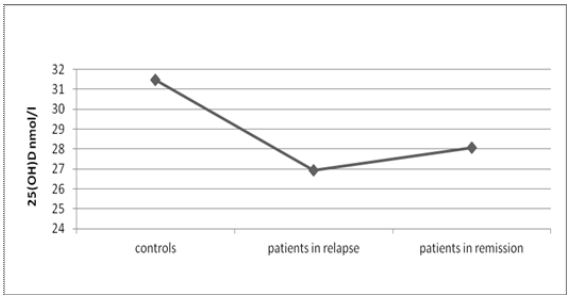

Figure 1. Serum levels of 25(OH)D

The assessment of the magnitude of the risk for developing MS, regarding exposure to the investigated factor showed a protective effect of Vitamin D with respect to the risk of disease occurrence (odds Ratio: 0.4125 relapse; 0.578 remission).

Negative statistically significant correlations between the concentrations of 25(OH)D both during relapse and remission and the degree of neurological deficit in the corresponding period were (Spearman's rho= -0.593, Sig = 0.03, during relapse; rho= -0.46, Sig=0.027, during remission). These results are presented on Fig. 2.

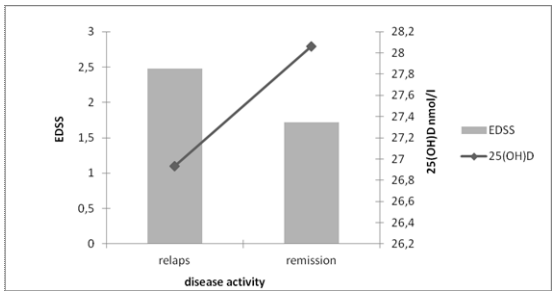

Figure 2. Changes in the degree of neurological deficit /EDSS/ and serum levels of 25(OH)D

Discussion

About 50% of the initially planned number of subjects has been recruited in the study so far and their results analyzed but we think that the trends and significant correlations found in these preliminary results are of scientific interest. The basic clinical parameters in the investigated patients – mean age, mean age of disease onset are in concordance with the last epidemiological study in Bulgaria [12]. In the healthy subjects we studied the serum levels of 25(OH)D were in the range indicating insufficiency, previously found in 75.8% of the Bulgarian population in a multicentre study of Borisova et al. in 2013 (6). The trend for lower serum 25(OH)D concentrations in healthy women, as compared to their male counterparts in the control group corresponded nicely to similar findings from that particular study [2]. Patients had even lower 25(OH)D, being on the edge of deficiency, as compared to the healthy subjects. Lower than 50 nmol. L^{-1} 25(OH)D serum levels were reported in 48-78% of the patients in different MS populations [4]. Previous observations on patients with relapse-remittent MS (RRMS) determined significantly lower serum levels of 25(OH)D and 1,25(OH) 2D in the relapse phase, as compared to remission, and in patients as compared to controls [8 ,9, 10]. At the current point of our study, this parameter does not differ significantly between the two disease periods. A limited number of clinical observations have confirmed the experimental results for sex differences in the metabolism and the immune-modifying action of 25(OH)D and 1,25(OH)2D [13, 14]. It has been proven that immune inflammatory alterations are dominant in females with RRMS, unlike males in whom the progress is more severe, with destructive T1 lesions on magnetic resonance imaging. The reduction in the number of IL17- and IFNγ – secreting cells and the increase in the regulatory CD4+ CD25+ Foxp3+ Lymph due to 1,25(OH)2D are more pronounced in females than males. These gender differences are supposedly modulated by estrogens [15, 16]. The small number of investigated subjects in the current study – 15 women and 8 men limits the possibility for interpretation of such differences. Mean 25(OH)D levels during relapse were close in both genders and are similar to those presented by Smolders et al. in 2008 [9]. The determined protective action of 25(OH)D regarding the risk of development of MS is in concordance with the clinical data for reduction of the risk with 41% for each increase of serum 25(OH)D with 50 nmol/L^{-1} [17].

The negative significant correlations of 25(OH)D levels in relapse and remission with the degree of disability in the respective periods that we established are quite indicative. They confirm the data presented by Van der Mei et al. in 2007, Soilu-Hannien et al. in 2008, and Neau et al. in 2011 [8, 10, 18]. Significantly lower serum levels of 25(OH)D in patients with primary and secondary progressive course of the disease, as compared with RRMS patients has been previously reported. It has been assumed that low 25(OH)D levels are related to the neurodegenerative alterations in patients with progressive MS phenotype [9]. Currently, unlike experimental data, the clinical studies do not present unambiguous proofs for the potential of Vit. D metabolites to reduce the clinical activity. Investigations in EAE found data for immune-modulating activity of 25(OH)D, 1,25(OH)2D. The therapy of 1,25(OH)2D inhibits the synthesis of TNFα, IFNγ, IL-17, increases the secretion of IL-4, stimulates the synthesis of TGFb1 and IL-10 from the regulatory populations, suppressing immune response [4, 19, 20].

Conclusion

The data about the protective action and the correlation between the changes in 25(OH)D and the degree of the neurological deficit give merits to further investigate the inclusion of this metabolite in the disease pathogenesis and the therapeutical potential for clinical activity control.

References

1. Milanov IG. [Multiple sclerosis and demyelinating diseases]. Sofia: Meditsina i fizkultura; 2010. Bulgarian.
2. Baranzini SE, Oksenberg JR. Role of genomics in studying MS. Int MSJ. 2002;9(3):91-8.
3. Pugliatti M, Sotgiu S, Rosati G. The worldwide prevalence of multiple sclerosis. Clin Neurol Neurosur. 2002;104:182-91.
4. Smolders J, Damoiseaux J, Menheere P, Hupperts R. Vitamin D as an immune modulator in multiple sclerosis, a review. J Neuroimmunol. 2008;194(1-2):7-17.
5. Szodoray P, Nakken B, Gaal J, Jonsson R, Szeqedi A, Zold E, et al. The complex role of vitamin D in autoimmune diseases. Scand J Immunol. 2008;68(3): 261- 9.
6. Borisova A-M, Boyanov M, Kolarov Z, Popivanov P, Shinkov A, Stoylov R, et al. [Recommendations for diagnosis, prevention and treatment of vitamin D deficiency]. Sofia: Bulgarian Society of Endocrinology; 2013. p. 9-20. Bulgarian.
7. Vankova M, Boyanov M. [Vitamin D insufficiency and its correction - a brief review of the literature]. Med Pregled. 2013;49(3):5-113. Bulgarian.
8. Neau JP, Artaud-Uriot MS, Lhomme V, Boundaud JY, Lebras F, Boissonnot L, et al. Vitamin D and multiple sclerosis. A prospective survey of patients of Poitou-Charentes area. Rev Neurol. 2011;167:317-23.
9. Smolders J, Menheere P, Kessels A, Damoiseaux J, Hupperts R. Association of vitamin D metabolite levels with relapse rate and disability in multiple sclerosis. Mult Scler. 2008;14:1220-4.
10. Soilu- Hanninen M, Airas L, Mononen I, Heikkilä A, Viljanen M, Hänninen A. 25-Hydroxyvitamin D levels in serum at the onset of multiple sclerosis. Mult Scler. 2005;11:266-71.
11. Jordanov BI. [Multiple sclerosis]. Sofia: Meditzina i sport-Sofia; 1989. p. 27-30. Bulgarian.
12. Topalov NL. [Clinical and epidemiological study of multiple sclerosis in Bulgaria] [dissertation]. Sofia: Medical University - Sofia; 1999. Bulgarian.
13. Barnes MS, Bonham MP, Robson PJ, Strain JJ, Lowe-Strong AS, Eaton-Evans J, et al. Assessment of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D3 concentration in male and female multiple sclerosis patients and control volunteers. Mult Scler. 2007;13(5):670-2.
14. Kragt JJ,van Amerongen B, Killestein J, Dijkstra C, Uitdehaaq B, Polman CH, et al. Higher levels of 25-hydroxyvitamin D are associated with a lower incidence of multiple sclerosis only in women. Mult Scler. 2009;1591):9-15.
15. Correale J, Ysrraelit MC, Gaitan MI. Gender Differences in 1,25 Dihydroxyvitamin D3 Immunomodulatory Effects in Multiple Sclerosis Patients and Healthy Subjects. J Immunol. 2010;185(8):4948-58.
16. Eikelenboom MJ, Killestein J, Kragt JJ, Uitdehaaq B, Polman CH. Gender differences in multiple sclerosis: Cytokines and vitamin D. J Neurol Sci. 2009; 286(1-2):40-2.
17. Munger KL, Levin LI, Hollis BW, Howards NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006;296(23):2832-8.
18. van der Mei IA, Ponsonby Al, Dwyer T, Blizzard L, Taylor BV, Kilpatrick T, et al. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. J Neurol. 2007:254(5):581-90.
19. Lysandropoulos AP, Jaquiéry E, Jilek S, Pantaleo G, Schluep M, Du Pasquier RA. Vitamin D has a direct immunomodulatory effect on CD8+ T cells of patients with early multiple sclerosis and healthy control subjects. J Neuroimmunol. 2011;233(1-2):240-4.
20. Peelen E, Knippenberq S, Muris AH, Thewissen M, Smolders J, Tervaert JW, et al. Effects of vitamin D on the pperipheral adaptive immune system: A review. Autoimmunity Rev. 2011;10(12):733-43.