Intradiscal levels of vitamin D and IgG in multiple sclerosis


Background – Intrathecal synthesis of IgG is a hallmark of multiple sclerosis (MS). Vitamin D may modulate B-cell function and dampen the synthesis of IgG. Objective – To investigate the relation between vitamin D levels in cerebrospinal fluid and serum and intrathecal synthesis of IgG. Methods – 25-hydroxyvitamin D (25(OH)D) and IgG were assessed in cerebrospinal fluid and serum in 40 patients with MS. Results – There was no significant correlation between the IgG index and 25(OH)D levels in cerebrospinal fluid or serum. The levels of 25(OH)D in cerebrospinal fluid and serum did not differ between patients with and without intrathecal synthesis of IgG. There was a non-significant trend towards a positive correlation between the concentrations of 25(OH)D and IgG in the cerebrospinal fluid, but not in serum. Conclusion – Physiological variation in vitamin D does not exert a major impact on intrathecal synthesis of IgG in MS.

Introduction

A poor vitamin D status is a risk factor for multiple sclerosis (MS) and possibly also for an unfavourable disease course (1, 2). Several studies have demonstrated that vitamin D modulates T-cell responses in MS (3, 4). Less is known about the impact of vitamin D on B cells. It has, however, been shown that the bioactive vitamin D metabolite, 1,25-dihydroxyvitamin D, suppresses B-cell maturation and IgG synthesis in vitro (5). Notably, a recent study reported a negative association between the concentrations of 25-hydroxyvitamin D (25(OH)D), which reflects the vitamin D status of the body, and IgG in serum of patients with cystic fibrosis (6). Intrathecal synthesis of IgG is an immunological hallmark of MS. Two previous studies have addressed the relation between the IgG index and serum levels of 25(OH)D in MS patients. One study reported an inverse correlation (7), whereas another did not (8).

Intrathecal synthesis of IgG is more likely to be influenced by the concentration of vitamin D in the cerebrospinal fluid than in the serum. In line with this notion, infiltrating immune cells in the CNS express CYP27B1, which converts 25(OH)D into 1,25-dihydroxyvitamin D (9), and 1,25-dihydroxyvitamin D synthesized locally in the CNS modulates the disease course in experimental autoimmune encephalomyelitis (10). We have previously reported that the CSF/serum ratio of 25(OH)D is below 1:100 and that it is lower in MS than in other neurological diseases (11). The aim of this study was to explore the relation between IgG and 25(OH)D in CSF in MS patients.

Materials and methods

Paired CSF and serum samples from 40 MS patients (26 women and 14 men; 36 relapsing–remitting, two secondary progressive, two primary progressive) were collected at the Neurological
Clinic, University of Ferrara. The mean disease duration was 35 (range 0–189) months. The four patients with progressive MS were not included in our previous report (11), but was included in this study because intrathecal synthesis of IgG is a typical feature also in these subtypes of MS. Ten CSF/serum pairs were collected in the winter, 16 in the spring, 11 in the summer and three in the fall. Written informed consent was obtained from all patients. The study was approved by the committee of research ethics at the University of Ferrara.

CSF and serum samples were collected at the same day and were kept frozen at −70°C until analysis. 25(OH)D in CSF and serum was measured with isotope dilution ultra performance liquid chromatography–atmospheric pressure chemical ionization–mass spectrometry as previously described (11). IgG and albumin levels were measured by immunochemical nephelometry with the Beckman Immage 800 System (Beckman Instruments, Fullerton, CA, USA). The IgG index was calculated according to the formula (CSF/serum IgG ratio)/(CSF/serum albumin ratio). An IgG index of 0.70 was considered as positive.

The data were not normally distributed, and distribution of variables was therefore compared with Mann–Whitney test, and Spearman r was used as a measure of association between two continuous variables. Statistical analyses were performed with Prism 4.0 software (GraphPad Software Inc., La Jolla, CA). Values are median (range) unless otherwise stated.

Results

The median concentrations of 25(OH)D were 0.26 (0.05–1.57) nm in CSF and 61.5 (14.0–132.0) nm in serum. The median CSF/serum albumin ratio was 4.9 (2.2–14.1) × 10⁻³, and the median IgG index was 0.65 (0.45–1.84). There was a non-significant trend towards a positive correlation between the levels of 25(OH)D and IgG in CSF (r = 0.28, P = 0.077), but not in serum (Fig. 1). There were no significant differences in the concentrations of 25(OH) D in CSF and serum between the 18 patients with IgG index > 0.70 and the 22 patients with IgG index < 0.70, or any significant correlation between the IgG index and the levels or the ratio of 25(OH)D in CSF or serum (Fig. 2).

Discussion

This is the first report on the relation between the intrathecal vitamin D levels and the intrathecal immune response in MS. Vitamin D reaches the CSF from the serum, and the CSF level of 25(OH)D is associated with the serum concentration and the blood–brain barrier integrity (11). In patients with MS, CSF IgG is derived either from local synthesis within the CNS or from the serum (12). We did not detect any correlation between CSF levels of 25(OH)D and the IgG index, suggesting that CSF levels of 25(OH)D do not exert any substantial effect on intrathecal IgG synthesis. It is therefore likely that the trend towards a positive correlation between the CSF concentrations of IgG and 25(OH)D is secondary to leakage of both 25(OH)D and IgG from serum to the CSF, rather than evidence of a primary association between the CSF concentration of 25(OH)D and the intrathecal synthesis of IgG.

There are several limitations to this study. First, the limited sample size does not allow us to exclude a weak association between intrathecal levels of vitamin D and IgG synthesis. Second, repeated lumbar puncture was not performed, which would have allowed us to address the association between seasonal fluctuation of 25-hydroxyvitamin D levels and IgG index in individual patients. Third, 1,25-dihydroxyvitamin D in CSF was not measured.
because the levels are below the detection limit of currently available assays. Vitamin D is generally believed to exert immunomodulatory effects through autocrine and paracrine effects of 1,25-dihydroxyvitamin D, and the concentration of 1,25-dihydroxyvitamin D in the microenvironment of antibody producing cells in the CSF and CNS may be poorly reflected by 25(OH)D in the CSF. The strength of the study is that 25(OH)D was measured in paired CSF and serum samples, thereby allowing us to address both the intrathecal and systemic vitamin D status in each patient.

In our study, an IgG index equal to 0.70 was chosen as reference value because this limit was assumed as cut-off value for an intrathecal IgG synthesis in the original paper of Tibbling and Link (13). The proportion of MS patients with a positive IgG index (18/40; 45%) was lower than the percentage usually observed (about 70%) (14), also at the University of Ferrara (15). This concurs with a relatively low proportion of patients with CSF-specific oligoclonal IgG bands (30/40; 75%) in our study, and is probably attributable to the selection and to the limited number of patients included.

There are several possible explanations why 25(OH)D was not associated with the IgG index. First, the concentrations of vitamin D metabolites in the CNS may be too low to affect IgG synthesis. In line with this idea, the mean concentration of 25(OH)D in CSF was 0.35 nm, whereas effect on B cells in vitro have been recorded at 250 nm 25(OH)D and 10 nm of 1,25-dihydroxyvitamin D (5). Second, the plasma cells or plasma blasts that synthesize IgG in the CSF could be relatively resistant to immunomodulators, including vitamin D. Third, the intrathecal synthesis of IgG in MS may be mediated by plasma blasts that are induced by T helper cells type 2 (Th2) (16). 1,25-dihydroxyvitamin D has been shown to induce Th2 cells (17), and high intrathecal levels could therefore possibly augment intrathecal IgG secretion.

We conclude that physiological variation in 25(OH)D levels in CSF and serum is not likely to exert a major effect on the intrathecal synthesis of IgG in patients with MS. This does not exclude that physiological variation in vitamin D levels may influence other aspects of B-cell function such as cytokine production and antigen presentation, or that pharmacological doses of vitamin D may affect intrathecal IgG synthesis.

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Conflict of interest

We have no conflict of interest.

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References


