Journal of Neuroimmunology 299 (2016) 118–123

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Serum 25-hydroxyvitamin D_3 is associated with disease status in patients with neuromyelitis optica spectrum disorders in south China

Yilong Shan [a,1], Lei Zhang [b,1], Sha Tan [a], Jianhua Huang [c], Xiaobo Sun [a], Yuge Wang [a], Wei Cai [a], Wei Qiu [a], Xueqiang Hu [a], Zhengqi Lu [a,*]

[a] Department of Neurology, The Third Affiliated Hospital of Sun Yat-sen University, No 600 Tianhe Road, Guangzhou City, China
[b] Department of Neurology, The Fifth Affiliated Hospital of Sun Yat-sen University, No 52 Meihuadong Road, Zhuhai City, China
[c] Department of Clinical Laboratory, The Third Affiliated Hospital of Sun Yat-sen University, No 600 Tianhe Road, Guangzhou City, China

ARTICLE INFO

Article history:
Received 31 March 2016
Received in revised form 22 August 2016
Accepted 12 September 2016
Available online xxxx

Keywords:
25-Hydroxyvitamin D3
Neuromyelitis optica spectrum disorder
Acute phase
Expanded Disability Status Scale

ABSTRACT

Here, we investigated the relationship between serum 25-hydroxyvitamin D_3 (25[OH]D_3) levels and neuromyelitis optica spectrum disorder (NMOSD). Patients with NMOSD had lower 25(OH)D_3 levels than healthy people, with lower levels in patients in the acute phase than those in remission. An inverse correlation was found between 25(OH)D_3 and Expanded Disability Status Scale scores of patients during attacks. Higher serum 25(OH)D_3 levels were associated with greater amelioration of symptoms during corticosteroid therapy. These results indicate that decreased vitamin D may be involved in NMOSD pathogenesis, and that 25(OH)D_3 serum levels may reflect the severity of NMOSD in the acute phase.

1. Introduction

Vitamin D_3, the major vitamin D form found in humans, is successively transformed into 25-hydroxyvitamin D_3 (25[OH]D_3) and 1,25-dihydroxyvitamin D_3 (1,25[OH]$_2D_3$) in the liver and kidney (Dimitrov and White, 2015). Owing to its immunoregulatory function, vitamin D is involved in different autoimmune diseases, such as multiple sclerosis (MS) (Munger et al., 2006), rheumatoid arthritis (Cutolo et al., 2006), and systemic lupus erythematosus (Muller et al., 1995). It has been reported that patients with MS have lower 25(OH)D_3 levels during attacks, and a poor 25(OH)D_3 status in these patients indicates a serious disease course (Smolders et al., 2008). Evidence shows that 1,25(OH)$_2D_3$ can not only inhibit the development of T helper (Th) 1 and Th17 and their migration to the central nervous system (CNS) (Mattner et al., 2000; Chang et al., 2010; Spanier et al., 2012) but also upregulate Th2 polarization (Sloka et al., 2011), which together lead to the amelioration of experimental autoimmune encephalomyelitis, a mouse model of MS. These findings are consistent with changes in the immune system of patients with MS (Alvermann et al., 2014). A similar protective effect of vitamin D was also found in patients with systemic lupus erythematosus and rheumatoid arthritis (Zwerina et al., 2011; Terrier et al., 2012). In addition, it has been shown in vitro that 1,25(OH)$_2D_3$ can directly affect B cells, through inhibiting B cell differentiation, and the antibody secretion of plasma cells (Chen et al., 2007).

Neuromyelitis optica spectrum disorders (NMOSD) are autoimmune diseases characterized by inflammatory demyelinating lesions in the CNS. NMO-IgG, an autoantibody that reacts with the water channel aquaporin 4, is a specific biomarker that is detected in the serum and CSF of patients with NMOSD (Wingerchuk et al., 2007). NMOSD patients with higher NMO-IgG titers and complement activation values show more serious clinical manifestations than those with lower titers (Takahashi et al., 2007; Jarius et al., 2008; Hinson et al., 2009). In addition, T cells and their related cytokines also participate in the development of NMO (Uzawa et al., 2014; Bettelli et al., 2006). Thus, the pathogenesis of NMOSD indicates that vitamin D can exert its immunoregulatory function in these disorders. However, only one study has reported that lower vitamin D serum levels were found in patients with NMOSD compared with levels in healthy controls (Min et al., 2014). Thus, our study aimed to provide additional evidence regarding the relationship between vitamin D and NMOSD.

2. Methods

2.1. Ethics statement

All participants gave written informed consent prior to participation in the study. This study was approved by the ethics committee of the Third Affiliated Hospital of Sun Yat-sen University.

* Corresponding author at: Department of Neurology, The Third Affiliated Hospital of Sun Yat-sen University, No 600 Tianhe Road, Guangzhou, Guangdong, China.
E-mail address: lzq1828@163.com (Z. Lu).
[1] Yilong Shan, Lei Zhang and Sha Tan, contributed equally to the manuscript.

http://dx.doi.org/10.1016/j.jneuroim.2016.09.006
0165-5728/

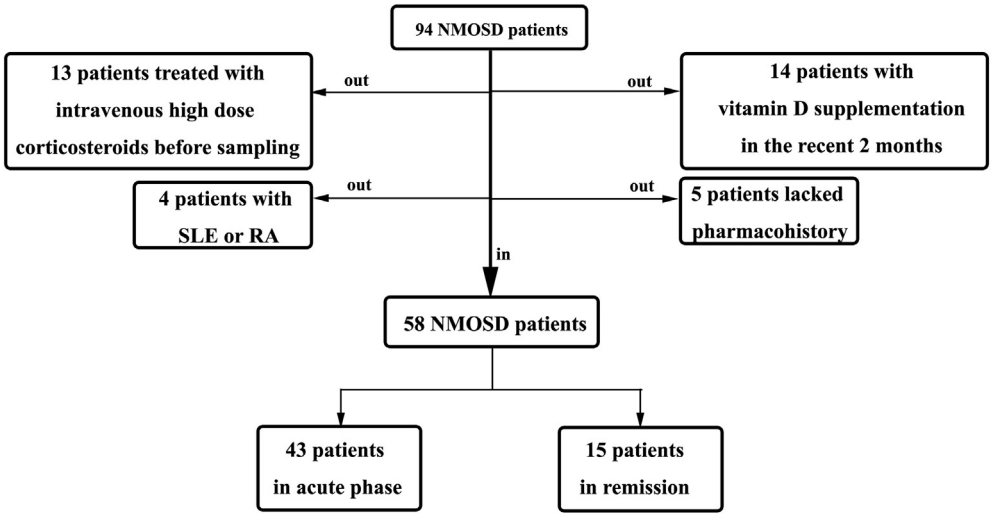

Fig. 1. Patients inclusion chart. Abbreviations: NMOSD, neuromyelitis optica spectrum disorders; SLE, systemic lupus erythematosus; RA rheumatoid arthritis.

2.2. Study participants

Patients with NMOSD enrolling in this study were collected retrospectively from January 1, 2013, to June 30, 2015, in the Department of Neurology of the Third Affiliated Hospital of Sun Yat-sen University. All patients diagnosed with NMOSD met the criteria established by Wingerchuk et al. (2007). The inclusion process is shown in Fig. 1. Demographic and clinical data, including sex, age of onset, age at sampling, season at sampling, disease duration (from initial symptoms to sample collection), annualized relapse rates (ARR), immunoglobulin (IgG, IgA, IgE), complement (C3, C4, CH50), were recorded at blood sampling. Expanded Disability Status Scale (EDSS) scores were assessed before and two weeks after intravenous high-dose corticosteroid therapy. An improved therapeutic effect was defined as a decrease in the patient's EDSS score of 1 or more 2 weeks after therapy (Ohno et al., 1987). Age-, sex-, and season-matched healthy participants from the Medical Examination Center of the Third Affiliated Hospital of Sun Yat-sen University served as the control group.

2.3. Serum vitamin D analysis

A total of 5 mL of venous blood was collected between 6:00 and 7:00 a.m. after an overnight fast on the second day after admission. All blood samples were collected prior to initiating corticosteroid treatment. The vitamin D levels in the serum samples were analyzed using a 25-Hydroxy Vitamin D EIA test kit (Immunodiagnostic Systems Limited, UK) according to the manufacturer's instructions. A serum level of $25(OH)D_3 < 50$ nmol/L was defined as $25(OH)D_3$ deficiency (Holick et al., 2011).

2.4. Aquaporin-4 IgG testing

The presence or absence of aquaporin-4 (AQP-4) IgG was determined in serum samples from patients with NMOSD and healthy participants using an anti-AQP4 transfected cell line in a commercial Biochip kit (Euroimmun, Lubeck, Germany) (Li et al., 2011). The results were recorded as positive or negative. All patients received the test.

2.5. Statistical analysis

All statistical analyses were conducted with SPSS version 13.0 software. Student's *t*-tests were used to evaluate continuous data with a normal distribution, whereas continuous data not normally distributed were analyzed using the Wilcoxon–Mann–Whitney *U* test. Pearson's correlation analysis was used to analyze correlations in normally distributed data, and analysis of covariance was used to distinguish the confounding factors. Partial correlation analysis was used to identify the association between EDSS scores and serum $25(OH)D_3$ levels without confounding factors. Multivariate logistic regression analysis was performed to identify factors independently associated with the disease status of patients with NMOSD. The nominal variables were expressed as N or percentage and compared using the chi-squared test. Probability

Table 1
Clinical characteristics of NMOSD patients in acute phase or in remission and healthy controls.

	NMOSD patients			Controls	*p*1	*p*2	*p*3	*p*4
	Acute phase n = 43	Remission n = 15	Total n = 58					
Gender (male:female)	4:39	3:12	7:51	14:102	0.37	0.63	0.40	1
Age of onset	37.40 ± 14.69	31.33 ± 10.84	35.83 ± 13.97	–	0.10	–	–	–
Age at sampling	40.16 ± 14.99	35.93 ± 11.23	39.07 ± 14.15	40.55 ± 14.67	0.26	0.88	0.24	0.53
Season at sampling (A:B:C:D)	13:13:7:10	6:5:0:4	19:18:7:14	8:36:14:28	0.59	0.81	0.66	1
EDSS score at sampling	4.81 ± 1.59	3.73 ± 1.74	4.53 ± 1.68	–	0.03	–	–	–
ARR at sampling	1.30 ± 1.8	0.88 ± 0.76	1.16 ± 1.61	–	0.17	–	–	–
AQP4-IgG positive (%)	40(93)	15(100)	55(94.8)	–	0.08	–	–	–
Oral prednisolone (%)	37(86)	12(80)	49(84.5)	–	0.59	–	–	–
Oral azathioprine (%)	16(37.2)	7(46.7)	23(39.7)	–	0.53	–	–	–

Abbreviations: NMOSD, Neuromyelitis optica spectrum disorders; A, spring; B, summer; C, autumn; D, winter; EDSS, Expanded Disability Status Scale; ARR, annualized relapse rate; AQP4, aquaporin 4.
p = comparison between two different groups; *p*1 = NMOSD patients in acute phase vs NMOSD patients in remission; *p*2 = NMOSD patients in acute phase vs healthy controls; *p*3 = NMOSD patients in remission vs healthy controls; *p*4 = NMOSD patients vs healthy controls.