Neurochemistry International 102 (2017) 89–94

Contents lists available at ScienceDirect

Neurochemistry International

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

Rapid communication

Low serum vitamin D levels and anti-*N*-methyl-D-aspartate receptor encephalitis: A case-control study

Yaqing Shu [a, 1], Qingmei Su [b, 1], Siyuan Liao [a], Tingting Lu [a], Rui Li [a], Xiaobo Sun [a], Wei Qiu [a], Yu Yang [a], Xueqiang Hu [a, **], Zhengqi Lu [a, *]

[a] Department of Neurology, The Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510630, China
[b] Department of Neurology, Yunfu People's Hospital, Yunfu 527300, China

ARTICLE INFO

Article history:
Received 2 July 2016
Received in revised form
29 October 2016
Accepted 7 November 2016
Available online 9 November 2016

Keywords:
25-hydroxyvitamin D
Anti-*N*-Methyl-D-aspartate receptor encephalitis
Biomarker
Immune effects

ABSTRACT

Background: Low vitamin D levels are associated with autoimmunity, but the relationship with anti-*N*-Methyl-D-aspartate receptor (anti-NMDAR) encephalitis is unknown.
Methods: 25(OH) D levels and clinical and cerebrospinal fluid parameters were evaluated in 30 patients with anti-NMDAR encephalitis and compared with 90 age-, sex-, and season-matched healthy controls.
Results: 25(OH)D levels were lower in patients with anti-NMDAR encephalitis compared to controls (43.89 ± 17.91 vs 64.24 ± 24.38 nmol/L, $p < 0.001$), especially for females (vs males, $p = 0.008$), aged $\leq$30 years (vs > 30 years, $p = 0.002$), severe impairment (mRS ≥ 5) (vs mRS < 5, $p = 0.018$), and limited treatment responses (vs favorable treatment, $p = 0.02$). Serum 25(OH)D levels were associated with age ($r = 0.393$, $p = 0.032$), and mRS ($r = -0.417$, $p = 0.022$).
Conclusions: Our data showed that serum 25(OH)D levels were reduced in patients with anti-NMDAR encephalitis.

1. Introduction

Anti-*N*-methyl-D-aspartate receptor (NMDAR) encephalitis is an immune-mediated disorder characterized by immunoglobulin G (IgG) antibodies against the GluN1 subunit of the NMDAR and presents with psychosis, seizures, encephalopathy, and cognitive and movement impairment (Dalmau et al., 2008; Hughes et al., 2010). This disorder can affect patients of all ages, but usually occurs in young women and children (Florance et al., 2009). Some patients have an ovarian teratoma, but the disorder may occur without tumor association. Most patients experience remarkable improvement after immunotherapy (Ishiura et al., 2008).

Vitamin D is synthesized from 7-dehydrocholesterol in the skin by the action of ultra violet light and to a limited extent from diet. Vitamin D is both a modulator of calcium homeostasis and immunity. Recently, the immuno-biological effects of vitamin D have received increasing attention. Vitamin D suppresses B cell proliferation and differentiation causing a decrease in immunoglobulin secretion as well as affecting T cell proliferation and maturation causing a decrease in the numbers of T cells with T helper (Th)1 and Th17 phenotypes (Boonstra et al., 2001). The major circulating form of vitamin D is 25-hydroxyvitamin D (25(OH)D) is measured to assess vitamin D status (Holick, 2007).

Low levels of vitamin D are associated with a variety of autoimmune disorders (AIDs) including multiple sclerosis (MS) (Salzer et al., 2012), recurrent inflammatory spinal cord disease (Mealy et al., 2012), neuromyelitis optica spectrum disorder (NMOSD) (Min et al., 2014), and other systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and insulin-dependent diabetes mellitus (IDDM) (Agmon-Levin et al., 2013). However, the importance of vitamin D in anti-NMDAR

Abbreviations: anti-NMDAR encephalitis, Anti-*N*-Methyl-D-aspartate receptor encephalitis; AIDs, autoimmune disorders; 25(OH)D, 25-hydroxyvitamin D; IgG, immunoglobulin G; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; IDDM, insulin-dependent diabetes mellitus; CTLs, healthy controls; CSF, cerebrospinal fluid; mRS, modified Rankin Scale; WBC, white blood cells; TP, total protein; Glu, glucose; CL, chlorine; MRI, brain magnetic resonance imaging; Gd-DTPA, gadopentetate dimeglumine; BMI, Body mass index.

* Corresponding author. Department of Neurology, The Third Affiliated Hospital, Sun Yat-sen University, 600 Tianhe Road, Guangzhou, Guangdong 510630, China.
** Corresponding author. Department of Neurology, The Third Affiliated Hospital, Sun Yat-sen University, 600 Tianhe Road, Guangzhou, Guangdong 510630, China.
E-mail addresses: huxueqiangqm@aliyun.com (X. Hu), lzq1828@aliyun.com (Z. Lu).

[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.neuint.2016.11.002
0197-0186/

encephalitis is unknown. Here, we analyzed 25(OH)D levels in anti-NMDAR encephalitis patients, and determined the association of vitamin D levels with clinical parameters in these patients.

2. Materials and methods

2.1. Patients and controls

This study recruited 30 patients with anti-NMDAR encephalitis (n = 30), and age-, sex-, and season-matched healthy controls (CTLs, n = 90). For each case, three control subjects were randomly selected and matched to the index case on age (±1), sex and season. All patients had been hospitalized from 1 August 2014 to 31 December 2015. All patients' serum and/or cerebrospinal fluid (CSF) were analyzed by indirect immunostaining using a commercially available kit (EUROIMMUN Medizinische Labordiagnostika, Lübeck, Germany) designed to detect an IgG antibody against NMDAR according to the manufacturer's instructions.

Season at blood sampling, defined as spring (March to May), summer (June to August), fall (September to November), and winter (December to February) was also acquired and matched to healthy controls.

Symptoms were categorized into the following nine groups: prodromal symptoms such as headache, fever, psychiatric symptoms, memory deficits, speech disturbances, seizures, movement disorders, loss of consciousness, sleep disorder, and central hypoventilation. Brain magnetic resonance imaging (MRI) and CSF examinations were reviewed. Individual or combined use of corticosteroids and intravenous immunoglobulins was defined as first-line immunotherapy, while administration of rituximab or azathioprine was defined as second line immunotherapy. The patients' neurological status was assessed using the modified Rankin Scale (mRS) (van Swieten et al., 1988). This study was approved by the ethics committee of the Third Affiliated Hospital of Sun Yat-sen University (No. [2015]2-175). All participants involved in this study provided written informed consent.

2.2. Vitamin D measurements

Serum 25(OH)D levels were measured with a commercially available Enzyme Linked Immunosorbent Assay kit (Immunodiagnostic Systems Limited, Bolton, UK) according to the manufacturer's instructions. Levels of 25(OH) D < 50 nmol/L were determined to be deficient and ≥50 nmol/L but < 75 nmol/L as insufficient (Holick, 2007).

2.3. MRI scanning

Brain MRI scanning was carried out for anti-NMDAR encephalitis patients using a GE 1.5T MR scanner (General Electric, Milwaukee, WI, USA). The slice thickness of axial scans was 5 mm. Conventional MRI protocols were previously described (Zhang et al., 2014). Gadopentetate dimeglumine (Gd-DTPA) was administered intravenously at a dose of 0.1 mmol/kg, and at about 15 min after contrast injection the T1-weighted sequence was repeated. Patients were considered to be active by MRI if there was one or more enhancing lesions in T1-weighted spin echo images after injection of Gd-DTPA.

2.4. Statistical analysis

The data were presented as the mean ± standard deviation (SD) [25(OH)D, age, BMI, CSF total protein (TP), CSF glucose (Glu), CSF chlorine (CL) levels] or median with range (mRS score, CSF white blood cell (WBC)).

As anti-NMDAR patients and CTLs were enrolled with 1:3 matched pair, the linear mixed effect model statistical test accounting for the pair information was used. In detail, "serum 25(OH)D level" as the dependent variable, "groups (anti-NMDAR patients or CTLs)" as the fixed variable, and "pair id" as the random variable were applied. At last, the difference between the model means (least square means) of two groups was estimated and tested.

In anti-NMDAR encephalitis subgroups, student *t* test was used to compare mean values of serum 25(OH)D levels. Correlations between serum 25(OH)D and age, BMI, mRS score, and CSF factors (CSF WBC, TP, Glu and CL) were analyzed by Spearman's rank test. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 16.0 software (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Demographic and clinical features of anti-NMDAR encephalitis patients and healthy controls

As shown in Table 1, a total of 30 anti-NMDAR encephalitis patients (mean age, 34.10 ± 16.17 years; mean BMI, 20.60 ± 2.75; female:male = 13:17; spring:summer:fall:winter = 8:9:6:7), and 90 CTLs (mean age, 35.32 ± 9.19 years; mean BMI, 21.99 ± 3.67; female:male = 39:51; spring:summer:fall:winter = 24:27:18:21) were included in our study. The median mRS in anti-NMDAR encephalitis patients was 3.0 (range, 1–5). Of 30 patients with anti-NMDAR encephalitis, 6 patients (20%) had prodromal symptoms (such as headache, fever), 17 patients (56.7%) had psychiatric symptoms, 3 patients (10%) had memory deficits, 4 patients (13.3%) had speech disturbances, 9 patients (30%) had seizures, 6 patients (20%) had movement disorders, 5 patients (16.7%) had loss of consciousness, 2 patients (6.7%) had sleep disorders, and 2 patients (6.7%) had central hypoventilation. Twenty-two patients (73.3%) received first line treatment such as corticosteroids or intravenous immunoglobulin, and 8 patients (26.7%) received second line treatments such as rituximab and azathioprine.

3.2. Comparison of serum vitamin D levels between patients with anti-NMDAR encephalitis and healthy controls

The mean concentration of serum 25(OH)D in patients with anti-NMDAR encephalitis was 43.89 ± 17.91 nmol/L compared with 64.24 ± 24.38 nmol/L in CTLs, (p < 0.001, Table 1). Among the 30 patients with anti-NMDAR encephalitis, 20 (66.7%) showed vitamin D deficiency (<50 nmol/L), 8 patients (26.7%) had vitamin D insufficiency (50–75 nmol/L), and only 2 patients (6.7%) had a sufficient vitamin D level (≥75 nmol/L). By contrast, 30 (33.3%) of 90 CTLs were considered vitamin D deficient, 39 (43.3%) had insufficient levels, and 21 (23.3%) having sufficient levels (Table 1).

Analysis according to gender demonstrated that 25(OH)D levels in females and male were significantly lower in patients with anti-NMDAR encephalitis than in CTLs (p < 0.001, p = 0.039, respectively) (Fig. 1A). According to season, 25(OH)D levels in the summer, fall, and winter were all significantly lower in patients with anti-NMDAR encephalitis than in CTLs (p < 0.001, p = 0.045, p = 0.048, respectively). Although serum 25(OH)D levels in the spring were also lower in patients with anti-NMDAR encephalitis than in CTLs, the difference was not significant (Fig. 1B). According to age, 25(OH)D levels in patients aged ≤ 30 years were significantly lower than that in CTLs aged ≤ 30 years (p = 0.001), while the difference between subgroup with age > 30 years was not statistical significance (Fig. 2A). According to BMI, patients with BMI ≤ 20 and BMI >20 both have significantly lower 25(OH)D levels