Vitamin D in amyotrophic lateral sclerosis

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Summary

Vitamin D supplementation has been proposed as a potential treatment to delay amyotrophic lateral sclerosis (ALS) progression. The aims of this study were to compare retrospectively vitamin D blood levels in ALS patients with those in healthy subjects; to correlate vitamin D blood levels with clinical functions in patients; and to evaluate whether administration of vitamin D could modify the clinical progression of the disease.
Vitamin D blood levels were evaluated in 57 ALS patients and in 57 healthy subjects.
In the ALS patients the following clinical variables were evaluated every 3 months: Medical Research Council scale (MRC) score; revised ALS functional rating scale (ALSFRS-R) score; forced vital capacity (FVC). Twenty-four patients were treated with high doses of cholecalciferol. No significant differences were found between the vitamin D blood levels in the ALS patients (18.8 ± 12.2) and the healthy subjects (20.7 ± 10.1). The vitamin D levels in the ALS patients did not correlate with recorded clinical parameters. No clinical differences in terms of ALSFRS-R, MRC or FVC were found between the treated and the untreated patients over time.
In ALS, as in other chronic neurological diseases, levels of vitamin D in blood appeared reduced, but no difference was found between the levels in ALS patients and in healthy subjects.
Oral vitamin D supplementation in ALS patients was not associated with better prognosis in comparison with untreated ALS patients. Further prospective controlled studies are needed to clarify the effect of vitamin D on the progression of ALS disease.

KEY WORDS: ALS, amyotrophic lateral sclerosis, cholecalciferol, vitamin D deficiency.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disease characterized by progressive degeneration of upper and lower motoneurons resulting in muscle weakness, paralysis and muscle atrophy, leading to death within 3-5 years of diagnosis (Hardiman et al., 2011).
Recent studies have evaluated the neuroprotective role of vitamin D in many neurodegenerative diseases (Shen and Ji, 2015a; Shen and Ji, 2015b; Burton and Costello, 2015; Miller et al., 2015; Balden et al., 2012). ALS patients show low levels of vitamin D, probably due to different factors: age, the presence of chronic disease, physical inactivity, reduced sun exposure, and malnutrition (Sato et al., 1997; Holick et al., 1989).
A recent study has proposed vitamin D supplementation as a potential treatment option to delay ALS progression (Karam et al., 2013). Different data have been published on an association between lower vitamin D levels and a faster rate of decline with shorter survival (Camu et al., 2014; Blasco et al., 2015). But the literature contains contradictory results on the association of vitamin D levels with clinical progression of ALS. In some studies, levels of vitamin D are associated with a better prognosis, in others with a worse prognosis (Karam et al., 2013; Camu et al., 2014; Blasco et al., 2015). Recent studies hypothesized a negative effect of vitamin D deficit on the structure and function of the neuromuscular junction, causing lack of a stress response in muscles (Gifondorwa et al., 2016). Moreover, the vitamin D receptor gene has been indicated as a possible candidate gene in muscle function (Bozsodi et al., 2016; Olsson et al., 2016).
The aims of this study were: i) to compare vitamin D blood levels in ALS patients with healthy subjects; ii) to correlate vitamin D blood levels with clinical and respiratory functions in enrolled patients; iii) to evaluate whether administration of vitamin D could modify the clinical progression of the disease.

Materials and methods

Patients

Fifty-seven patients (47 men and 10 women; mean age 63 ± 9.9 years, range 41-82 years) with definite or probable ALS and 57 healthy subjects (29 men and 28 women, mean age 62.1 ± 11.2) were enrolled. The study was approved by the medical ethics committee of the Umberto I Hospital in Rome and was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. All patients gave their written informed consent prior to their inclusion in the study. The inclusion criterion for ALS patients was a diagnosis of definite or probable ALS according to the revised El Escorial criteria (Brooks et al., 2000).
Demographic and clinical characteristics at baseline are described in Table I.

Study design

This was a retrospective study. We revised the folders of ALS patients regularly seen at our center, evaluating the patients found to have low or normal levels of vitamin D in their blood. Only ALS patients with low levels of vitamin D received high doses of cholecalciferol (100,000 I.U.), which is the treatment commonly performed in clinical practice. The demographic and clinical characteristics of these patients were matched with those of a group of ALS patients with similar characteristics but with normal levels of vitamin D. Moreover, the levels of vitamin D in the ALS patients were compared with those found in healthy subjects.

Comparison of vitamin D blood levels in ALS patients and healthy subjects

We correlated the vitamin D blood levels of the ALS patient group and the group of healthy subjects using the following clinical variables: ALS Functional Rating Scale-Revised (ALSFRS-R) score, Medical Research Council (MRC) scale for testing muscle strength, and forced vital capacity, expressed as percent predicted (FVC %).
The night prior to the baseline examination, the participants completed an overnight (12-hour) fast, except for taking their regularly prescribed medications. Blood samples were drawn into chilled tubes containing ethylenediaminetetraacetic acid; plasma was aliquoted and stored at -70°C until the examination. We used a Roche COBAS E 601 spectrometer (Roche Diagnostics International Ltd CH-6343 Rotkreuz Switzerland) to measure 25-hydroxyvitamin D3 and D2 levels. With regard to the vitamin D readings, levels below 20 ng/dl were considered insufficient, while values of 20-30 ng/dl indicated a deficiency, and values higher than 30 ng/dl were taken as normal levels.
The ALSFRS-R is a validated measure of functional impairment in ALS (Cedarbaum et al., 1999). It is a questionnaire containing 12 items rated from 0 (complete dependence for that function) to 4 (normal function), divided into 3 sub-scores (bulbar 12, motor 24, and respiratory 12).
Muscle strength of limbs was assessed using the MRC scale, an ordinal scale ranging from 0 (absence of movement) to 5 (contraction against full resistance) that quantifies muscle weakness in isolated muscles or muscle groups (Gregson et al., 2000).
Spirometry was carried out using the Inspire PRO 5.8 spirometer (Mir Medical International Research Srl, Rome, Italy). FVC (L) and forced expiratory volume in first second (FEV1) (L/s) were analyzed. The highest values were used in the analysis.

Comparison of ALS patients treated with vitamin D and untreated ALS patients

Twenty-four ALS patients (15 men and 9 women) were treated with high doses of cholecalciferol (100,000 I.U. per week for four weeks and then 25,000 I.U. every 15 days). The data collected were compared with those of 24 untreated ALS patients (7 women and 17 men).
All patients were treated with riluzole 100 mg/day, according to the current international ALS guideline. Age at onset was taken as the time at which motor weakness was first noted by the patient.
Disease duration was expressed as the time from the first symptom to the day of the beginning of the therapy. All the patients were submitted to ALS-FRS-R, MRC, and FVC evaluation at baseline (B), at 3 months (T1) and at 6 months (T2) after starting the treatment. During each visit we investigated the presence of side effects related to hypercalcemia (calcium serum levels higher than 10.5 mg/dl), such as hematuria, gastrointestinal symptoms, lethargy or cardiac palpitations.

Data analysis

We used paired Student's t-test to evaluate differences in vitamin D blood levels between the ALS patients and the healthy subjects.
Chi-square test was used to compare qualitative values. Pearson's score (P) was performed for the correlation between vitamin D levels and ALS-FRS-R score, MRC score and FVC. Repeated measures ANOVA was applied to trace the level of clinical variables over time in treated and untreated patients. The Bonferroni post hoc test was applied in the event of significance at ANOVA. For all tests, values of $p < 0.05$ were considered statistically significant.

Results

Comparison of vitamin D blood levels in ALS patients and healthy subjects

Vitamin D levels in patients did not correlate with demographic features (P=–0.6; p=0.66), ALSFRS-R (P=0.10; p=0.44), MRC (P=–008; p=0.64), FVC (P=0.07, p=0.57), and between spinal or bulbar ALS (p=0.90). No statistical differences were found between the ALS patients and the healthy subjects in terms of demographic and baseline clinical characteristics (Tab. I).

Table I - Main characteristics of ALS patients and healthy subjects. Values are reported as mean (± standard deviation) or percentage.

	ALS patients	Healthy subjects	p-value
Total	57	57	
Sex n (%)			
Male	47 (82.4%)	29 (50.8%)	
Female	10 (17.5%)	28 (49.1%)	
Age (years)	63.0 ± 9.9	62.1 ± 11.2	ns
Vitamin D (ng/dl)	18.8 ± 12.1	20.7 ± 10.1	ns

Thirty-four ALS patients (59.6%) showed vitamin D levels lower than 20 ng/dl, 18 (31%) had vitamin D levels between 20 and 30 ng/dl, and only 5 patients (8.8%) had normal values. In the control group, 28 (49%) had insufficient vitamin D levels, 15 (26.3%) had a deficiency, and 14 (24.6%) had optimal levels. No significant variation in vitamin D levels between the two groups was found (p=0.369).

Comparison of ALS patients treated with vitamin D and untreated ALS patients

The baseline characteristics of 24 patients treated with vitamin D were compared with those of 24 untreated patients. No statistical differences in demographic and clinical features were found between the vitamin D-treated ALS patients and the untreated ALS patients (Tab. II). Over time, no differences in ALSFRS-R score, MRC score or FVC were found between the two groups (Tab. III; Figs. 1-3). Vitamin D levels were statistically different between the two patient groups (56 ± 10 in the treated group and 21 ± 15 in the untreated group; p=0.01) only at T2.
The patients treated with high-dose cholecalciferol showed no side effects or adverse events.

Discussion

In this study we evaluated vitamin D blood levels in a group of ALS patients and in matched healthy subjects, expecting to find differences. We found a high incidence of vitamin D deficiency both in patients with ALS and in control subjects, without significant differences between the two groups. The vitamin D deficiency was found to be objectively lower than expected. Moreover, no relationship between low levels of vitamin D and clinical variables (ALSFRS-R score, MRC score and FVC) was found, and high-dose vitamin D treatment did not result in any clinical improvement over untreated patients.

In the past decade, interest in the role of vitamin D deficiency in various diseases of the nervous system has increased (Suzuki et al., 2012; Buell et al., 2010; Balion et al., 2012; Mowry et al., 2012). In other neurological diseases, low vitamin D levels have been found to be associated with greater disease severity and with a worse prognosis (Shen and Ji, 2015a. Shen and Ji, 2015b; Burton and Costello, 2015; Miller et al., 2015; Balion et al., 2012; Mowry et al.; 2012). This interest is partially due to convincing data, *in vitro* or in animal models, showing a positive role of vitamin D in the development of the nervous system, in neuroprotection, and in the modulation of neuroinflammation (Gianforcaro and Hamadeh 2012; Solomon et al., 2011). Several studies have analyzed vitamin D status in neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease (Buell et al., 2010; Gianforcaro and Hamadeh, 2012). Recent studies on the G93A transgenic mouse model of ALS showed improved functional outcome compared to control mice after supplementation of vitamin D (Gianforcaro and Hamadeh, 2012, Solomon et al., 2011; Gianforcaro et al., 2013). Moreover, vitamin D applied on motoneurons cultured *in vitro* seems to potentiate the effects of glial- and brain-derived neurotrophic growth factors, protecting cells from Fas-induced cell death (Camu et al., 2014). Although ev-
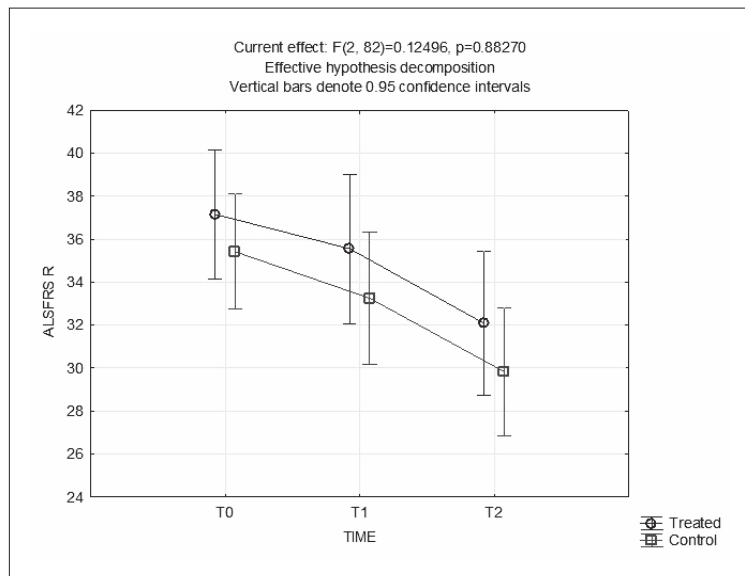

Figure 1 - ALSFRS-R total score in ALS patients treated with vitamin D and in untreated ALS patients. ALSFRS-R: ALS functional rating scale revised.
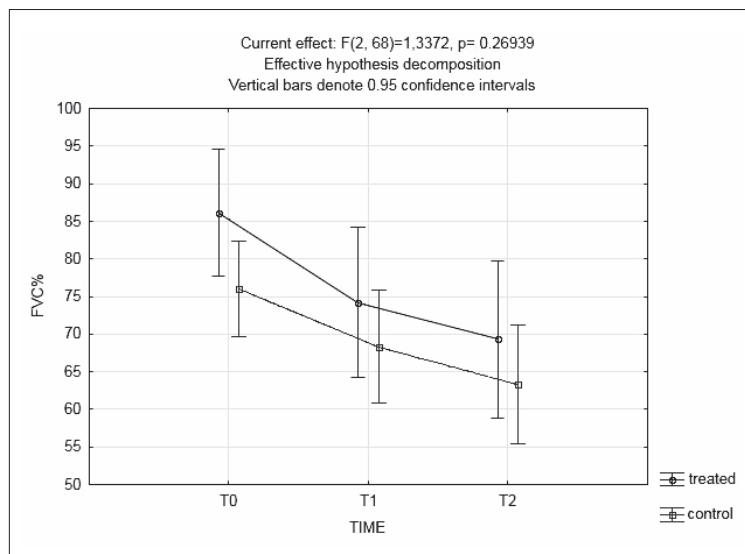

Figure 2 - FVC value in ALS patients treated with vitamin D and in untreated ALS patients.

idence from the animal models suggests an effect of supplemental vitamin D on ALS, these positive results may not always extend to humans (Minshull et al., 2016; Blasco et al., 2015; Yang et al., 2016). Similarly to our results, studies in healthy subjects indicated that vitamin D intake did not improve muscle tropism and strength (Agergaard et al., 2015). The ability of the neuromuscular system to remodel in response to resistance rehabilitation training or following injury still remains to be understood (Minshull et al., 2016).

Nevertheless, other Authors reported a high incidence of vitamin D deficiency in patients with ALS, and also found, at the 9-month follow-up, a slower decline in ALS-FRS-R scores in 20 patients treated with 2,000 IU of cholecalciferol per day compared with patients who did not take the same treatment (Karam et al., 2013). Camu et al. (2014) found an association between low vitamin D levels and a shorter survival time in patients with ALS, even after excluding non-ambulatory patients with vitamin D deficiency, probably due to severely decreased physical activity (Camu et al., 2014). Other studies revealed that high vitamin D levels correlate with a worse prognosis in ALS patients (Blasco et al., 2015). The discrepancy between these data and ours might be because ALS patients can not perform adequate physical activity, and despite the vitamin D supplementation, they do not get those benefits observed in physically active patients (Al-Eisa et al., 2016; Agergaard et al., 2015). Recently, Al-Eisa et al. evidenced a correlation between higher serum vitamin D levels and better physical muscle performance in physically active healthy older adults, probably by regulate on of the biosynthesis of creatine kinase, lactic acid dehydrogenase, troponin I, and hydroxyproline (Al-Eisa et al., 2016). Moreover, vitamin D receptor gene expression could have a role in muscle function, with a possible effect of this vitamin on proliferation and differentiation of muscle precursors (Bozsodi et al., 2016, Olsson et al., 2016). Some studies on the effect of this gene's variants on muscle strength are still contradictory, but individual genetic patterns might explain the inconsistency of our results on the association between vitamin D and muscle function (Bozsodi et al., 2016).

Certainly, our study has limitations. Its retrospective nature could have caused a selection bias, and the relatively small sample size did not allow some statistical analyses or stratification for certain variables. Also, even though our results showed that patients receiving vitamin D supplementation did not present any clinical improvement, the detection of a vitamin D deficit in ALS patients should be always highlighted because it could be as-
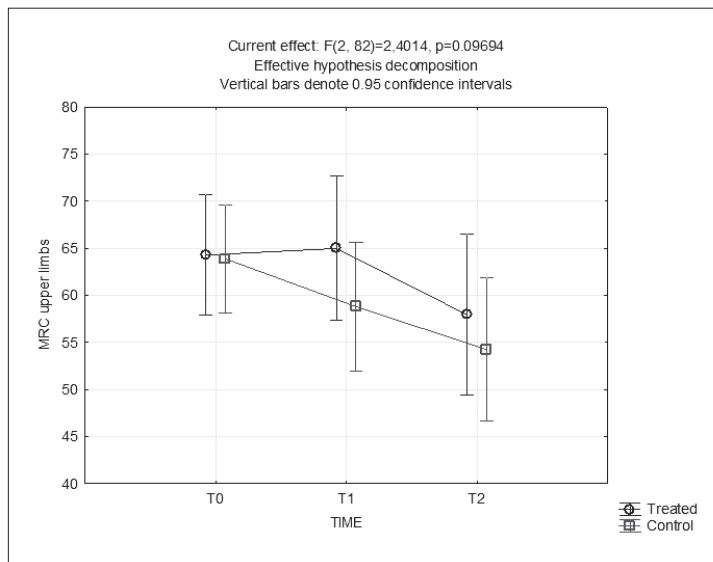

Figure 3 - MRC score of upper limbs in ALS patients treated with vitamin D and in untreated ALS patients. MRC: Medical Research Council.

Table II - Baseline clinical variables of ALS patients treated with vitamin D and untreated ALS patients. Values are reported as mean (± standard deviation) or percentage.

	Treated patients	Untreated patients	p-value
Total	24	24	
Sex n (%)			ns
Male	15 (62.5%)	17 (70.8%)	
Female	9 (37.5%)	7 (29.2%)	
Phenotype			ns
Bulbar	7 (29.1%)	10 (41.6%)	
Spinal	17 (70.8%)	14 (58.3%)	
Age (years)	63.2 ± 9.9	64.2 ± 11.2	ns
Disease duration	2.4 ± 1.6	2.2 ± 1.7	ns
ALSFRS-R	36.6 ± 7.5	35.4 ± 6.0	ns
FVC%	91 ± 14	70 ± 5	ns
MRC Upper limbs	64.8 ± 14.6	63.8 ± 12.9	ns
MRC Lower limbs	60.4 ± 14.2	55.4 ± 16.1	ns
Vitamin D (ng/dl)	18.8 ± 15.5	20.8 ± 16	ns

Abbreviations: MRC: Medical Research Council scale; ALSFRS-R: ALS functional rating score revised; FVC: forced vital capacity.

Table III - Clinical variables over the time in ALS patients treated with vitamin D and untreated ALS patients. Values are reported as mean (± standard deviation).

	Treated patients			Untreated patients		
	Baseline	T1	T2	Baseline	T1	T2
ALSFRS-R total	36.6 ± 7.5	35.9 ± 7.9	32.0 ± 8.3	35.4 ± 6.0	33.2 ± 6.8	29.8 ± 6.1
MRC Upper limbs	64.8 ± 14.6	62.7 ± 20.0	57.9 ± 19.8	63.8 ± 12.9	58.8 ± 17.4	54.2 ± 17.3
MRC Lower limbs	61.6 ± 14.3	60.7 ± 14.1	57.2 ± 13.1	55.4 ± 10.3	55.8 ± 11.4	51.8 ± 14.3
FVC%	75.9 ± 22.0	69.2 ± 21.8	68.9 ± 21.0	73.8 ± 18.3	68.3 ± 16.7	63.3 ± 17.3

Abbreviations: MRC: Medical Research Council scale; ALSFRS-R: ALS functional rating score revised; FVC: forced vital capacity.

sociated with a delay in disease onset and reduced disease severity, as demonstrated in previous studies (Karam et al., 2013, Camu et al., 2014, Solomon et al., 2011). Further studies are certainly needed to clarify whether a relationship truly exists between vitamin D levels and prognosis in ALS. It is still not possible to draw a definite conclusion regarding the role of vitamin D supplementation in ALS patients. However, our study adds information to the literature on the correlation between ALS and low levels of vitamin D.

In conclusion, in ALS, as in other chronic neurological diseases, levels of vitamin D in blood appeared reduced but without significant differences emerging compared with healthy control subjects. Moreover, no relationship between ALS disease and low vitamin D levels was confirmed in our study. Any benefit of supplementing vitamin D in ALS patients remains to be well defined, but the present study did not show any benefit: supplementation of high-dose oral vitamin D in ALS patients was not associated with better prognosis in comparison with untreated ALS patients. A prospective controlled study, with a larger sample size and a longer follow-up period, is needed to evaluate the effect of vitamin D on the progression of disease and the possible role of its use as a supplement in ALS patients.

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