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ORIGINAL ARTICLE

Preventive effect of vitamin D3 supplementation on conversion of optic neuritis to clinically definite multiple sclerosis: a double blind, randomized, placebo-controlled pilot clinical trial

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Abstract Multiple sclerosis (MS) presents with optic neuritis (ON) in 20 % of cases and 50 % of ON patients develop MS within 15 years. In this study, we evaluated the preventive effects of vitamin D3 administration on the conversion of ON to MS (primary outcome) and on the MRI lesions (secondary outcome) of ON patients with low serum 25 (OH) D levels. Thirty ON patients (15 in each of 2 groups, aged 20–40 years) with serum 25 (OH) D levels of less than 30 ng/ml were enrolled in a double blind, randomized, parallel-group trial. The treatment group (cases) received 50,000 IU of vitamin D3 weekly for 12 months and the control group (controls) received a placebo weekly for 12 months. Finally, the subsequent relapse rate and changes in MRI plaques were compared between the two groups. Risk reduction was 68.4 % for the primary outcome in the treatment group (relative risk = 0.316, $p = 0.007$). After 12 months, patients in the treatment group had a significantly lower incidence rate of cortical, juxtacortical, corpus callosal, new T2, new gadolinium-enhancing lesions and black holes. The mean number of total plaques showed a marginally significant decrease in the group receiving vitamin D3 supplementation as compared with the placebo group ($p = 0.092$). Administration of vitamin D3 supplements to ON patients with low serum vitamin 25 (OH) D levels may delay the onset of a second clinical attack and the subsequent conversion to MS.

Keywords Multiple sclerosis · Optic neuritis · Vitamin D3 · 25 (OH) D · Clinical trial · Isfahan, Iran

Clinical trial registration number: IRCT201205319919N1.

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Introduction

Acute onset clinically isolated syndrome (CIS) is the first manifestation of multiple sclerosis (MS) in approximately 90 % of patients. Optic neuritis (ON), brainstem, and spinal cord syndromes are the most prevalent types of CIS [1]. Optic neuritis most commonly presents with sub-acute painful visual loss [2], and may present as an isolated episode or as the initial presentation of MS [3, 4]. Twenty percent of MS patients present with ON, and 50 % of isolated ON cases convert to MS within 15 years [2].

The presence of silent brain MRI lesions (especially when accompanied by spinal cord lesions) at the time of

ON diagnosis is the most accurate indicator of the likelihood of a patient going on to develop MS [3]. The 5-year risk for developing clinically definite multiple sclerosis (CDMS) in patients with three or more silent white matter brain MRI lesions has been reported to be as high as 51 % compared with 16 % in patients with normal brain MR imaging [5].

Our question was whether vitamin D3 supplementation in patients who had experienced a single episode of clinically isolated optic neuritis could prevent or delay a relapse, and thus reduce the risk of conversion to CDMS.

Recently, the immunomodulatory effects of vitamin D3 in MS have been endorsed. In addition, some limited studies have revealed that vitamin D3 supplements are both safe and efficacious when used on patients with established MS [6–10]. Assessment of patients' vitamin 25 (OH) D status at the time of their first demyelinating attack can assist in analyzing whether it is a reliable predictor of subsequent attacks. Up until now, primary prevention trials to evaluate the effect of serum 25 (OH) D optimization in reducing the risk of MS development have not been performed on humans [6].

We hypothesized that the correction of low serum 25 (OH) D levels by administering oral vitamin D3 to patients with concurrent CIS would delay the onset of CDMS. In this double-blind, randomized clinical trial, we aimed at determining the efficacy, during a 12-month follow-up period, of vitamin D3 supplementation [serum 25 (OH) D optimization] in preventing the development of MS in ON patients. We also investigated the effects of vitamin D3 supplementation on the MRI plaques of ON patients in the treatment group, as compared with those of the placebo group, in more detail than has previously been undertaken in similar studies on CDMS patients.

Materials and methods

Design and sample

This was a double-blind, randomized, parallel-group trial carried out on ON patients who had been diagnosed at Al-Zahra and Feiz Ophthalmology Referral Centers between June and October 2010, in Isfahan province, Iran. Primarily, the study enrolled 37 patients who had been diagnosed with definite optic neuritis, as confirmed through history-taking, physical examination, and MRI scanning. These patients had the triad of sub-acute unilateral loss of vision, periocular pain, and impaired color vision. At the end of October 2010, 30 ON patients who met the entry criteria were randomly assigned to either the placebo group or the vitamin D3 treatment group. Randomization was done using permuted-block randomization in blocks of two.

Inclusion criteria were as follows: (1) male and female ON patients within the age range 20–40 years, (2) non-fulfillment of the 2005 revised McDonald criteria for MS [11] at the time of inclusion in the study, (3) a serum 25 (OH) D level of less than 30 ng/ml, and (4) seronegativity for anti-aquaporin 4 and other autoimmune antibodies.

Exclusion criteria were: (1) patients who had consumed vitamin D3 supplements at a level higher than 400 IU/day before the onset of ON, (2) patients who had used any medication that could influence the prognosis of ON (e.g., β-interferon) during the 12-month follow-up period, (3) women who were pregnant or breast feeding, (4) patients with kidney or liver dysfunction, (5) patients who demonstrated poor compliance with instructions to take vitamin D3 or the placebo, or who failed to attend for follow-up visits, MRI scanning, and 25 (OH) D measurements during the study, (6) patients with a history of nephrolithiasis, and (7) patients suffering from concurrent granulomatous diseases [7].

The study was undertaken in compliance with the declaration of Helsinki and standards of good clinical practice according to the International Conference on Harmonisation of Technical Requirements for registration of pharmaceuticals for human use. This trial was approved by the Ethics Committee of Isfahan University of Medical Sciences and all patients gave informed consent. The clinical trial was registered with the Iranian Registry of Clinical Trials (IRCT201205319919N1) (Fig. 1).

The following processes were carried out consecutively for each patient included in the study at the time of ON diagnosis: (1) measurements of serum 25 (OH) D level, anti-aquaporin 4 antibody, anti-nuclear antibody, anti-double stranded DNA antibody, anti-phospholipid antibody, anti-β2-glycoprotein, and angiotensin-converting enzyme antibody, (2) performance of brain and spinal MRIs, (3) administration of an intravenous single daily dose of methylprednisolone (1 g/day) for 3 days, followed by oral prednisolon, and (4) administration of vitamin D3 at a dosage of 50,000 IU/week in the form of oral pearls of vitamin D3 (drug A) to group A (treatment group), and a similar pearl-shaped placebo (drug B) to group B (control group).

Patients and investigators were kept blinded to the randomized allocation of the placebo/vitamin D3 treatment throughout the trial. The two groups were followed up for a period of 12 months from their inclusion time.

The follow-up phase was carried out on the basis of regular monthly visits. Patients' serum 25 (OH) D and calcium levels were measured monthly so that we could prevent toxicity if levels were too high. Serum 25 (OH) D was measured using a commercially available radioimmunoassay kit (DiaSorin, Stillwater, MN, USA). Serum

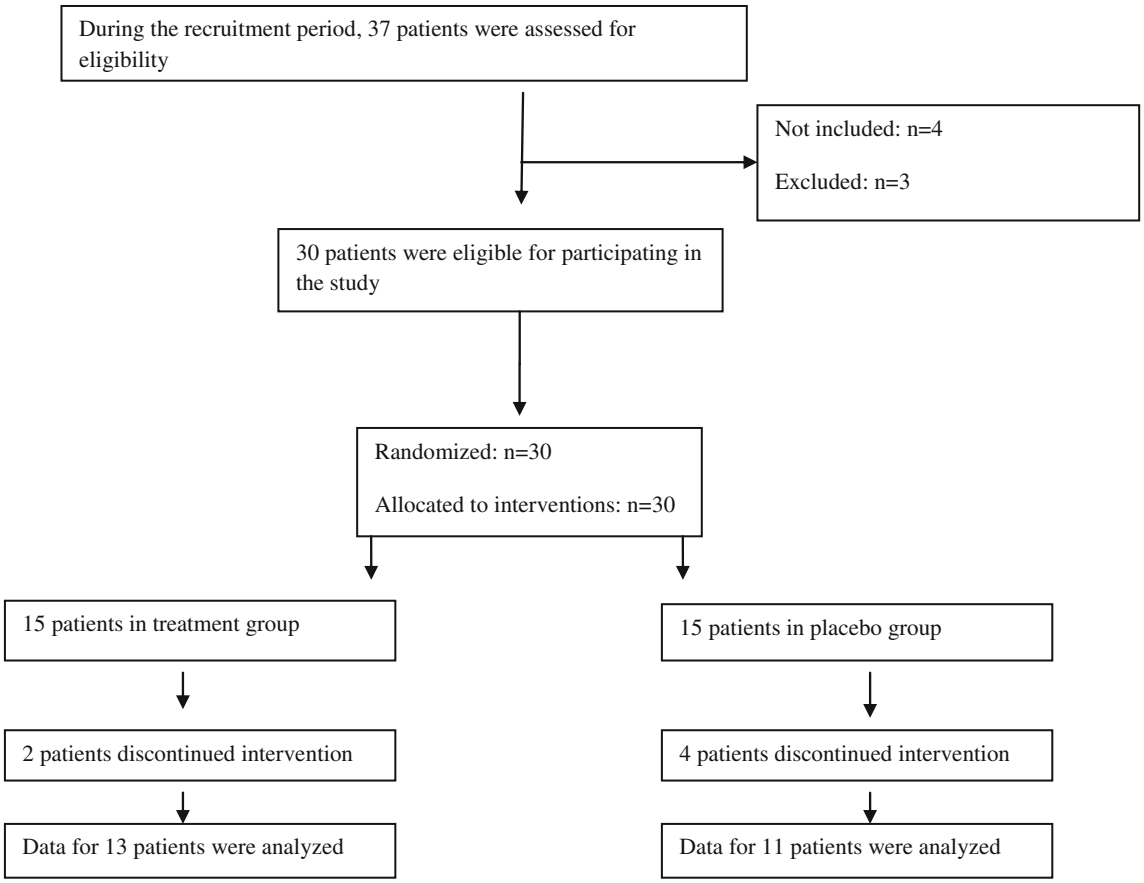

Fig. 1 Design of the trial

calcium was measured using Architect C1600®Abbott by standard auto-analyzer technique.

We discontinued high-dose vitamin D3 supplementation in patients when their serum 25 (OH) D level reached a normal upper limit (100 ng/ml). Subsequently, serum 25 (OH) D status was maintained in these patients at a normal upper limit using a lower dose of vitamin D3. Brain and spinal MRIs were carried out for both groups at baseline after 6 months and after the 12-month follow-up period. We used a standard 1.5-T MRI protocol with a 5-min scanning time delay after the injection of a single dose of 0.1 mmol/kg gadolinium (axial and sagittal T1-, T2-weighted and FLAIR images, 5-mm slice thickness). All patients were tracked closely for any new neurologic symptoms or relapses.

Outcome variables

The primary outcome in this clinical trial was ON conversion rate to MS in the two groups. The pre-specified secondary outcomes were as follows: changes in the number of T1-hypointense and T2-hyperintense brain MRI lesions at various sites and also the number of new T2- and gadolinium-enhancing lesions in both groups after 12 months. The patients' MRIs were assessed by a neuroradiologist blinded to both the randomized allocation and the nature of the intervention.

Statistical analysis

Statistical analysis was performed using SPSS software version 20 (SPSS Inc., Chicago, IL, USA). Quantitative variables are presented as either mean ± SD (median and range), and categorical variables are given as numbers and percentages. The differences between the groups as regards both the quantitative variables and categorical variables were assessed using Mann–Whitney *U* and Chi-squared tests. The difference between groups in terms of the mean total number of plaques, adjusting for its baseline values after normalizing with logarithmic transformation, was assessed using ANCOVA. Also, the incidence-rate ratio for secondary outcomes at the end of the 12-month follow-up, controlling for baseline characteristics (as appropriate), was calculated using Poisson

regression. A p value of less than 0.05 was considered as statistically significant.

Results

Four patients in the control (placebo) group and two in the case (treatment) group were excluded after 3 months due to poor cooperation including failure to attend for follow-up visits, lack of compliance in taking medication, and non-attendance for blood sampling. Finally, 13 cases and 11 controls cooperated until the end of study. The female:male ratio in the treatment group and control group was 12:1 and 10:1, respectively. The mean ± SD age of cases and controls was 26.23 ± 5.71 and 24.55 ± 4.50, respectively ($p = 0.44$). Serum levels of 25 (OH) D were optimized only for patients in the treatment group through the administration of vitamin D3 supplements, and the controls were given a similar-looking placebo.

None of the cases were affected by vitamin D toxicity or hypercalcemia during the study. The mean level of 25 (OH) D in cases and controls at the time of optic neuritis diagnosis was 13.7 ± 6.24 (14 [5–25]) and 16.42 ± 5.10 (14.9 [6.3–23.2]), respectively ($p = 0.302$). During the 12-month follow-up, five patients in the placebo group (45.5 %), and none of the patients in the vitamin D3 group, experienced a second demyelinating attack [$\chi^2 = 7.15$, relative risk (RR) = 0.316; $p = 0.007$], implicating the protective effect of vitamin D3. Also, absolute risk reduction was 45.5 % and the number need to treat was 2.2 (rounded to 2), accordingly. The mean serum level of 25 (OH) D in the five patients who developed MS was 17.8 ± 4.11 (16.2 [13.5–22.5]) at the time of ON diagnosis and 18.49 ± 3.72 (17.52 [14.23–23.2]) ($p = 0.781$) at the time of MS diagnosis (the second demyelinative attack). The difference in the mean level of 25 (OH) D at the time of ON diagnosis in the controls who developed MS, in comparison with the controls who did not develop MS (15.57 ± 5.73 (15.2 [6.3–23.2]), was not statistically significant ($p = 0.65$).

There was no relationship between serum 25 (OH) D levels and the number of MRI plaques at the time of ON diagnosis ($r = -0.02$, $p = 0.92$). Table 1 shows the mean ± SD and median (range) of demyelinative plaques in the MRIs of patients at baseline and at the end of the study period. Also, the incidence rate-ratio (95 % CI) for each outcome considered at the final stage of the study has been presented (Table 1).

As can be seen from Table 1, the incidence rates of black holes, cortical, juxtacortical, corpus callosal, new gadolinium-enhanced, and new T2 lesions were significantly lower in the treatment group than in the placebo group, controlling for their baseline values. These figures represent the ratio of the rate at which the events of interest occurred among patients who received vitamin D3 supplementation compared with those who did not. Thus, the key feature exhibited by the analysis of data using Poisson regression is that the rate at which patients experienced the studied outcomes, which were adjusted for their baseline, is significantly lower after 25 (OH) D optimization. For instance, the incidence rate of black holes in the treatment group is 84 % (i.e. 1–0.16) less than that in the control group ($p = 0.001$).

An overall comparison of the mean number of total MRI-detected plaques in various regions of the brain using ANCOVA, controlling for the baseline difference, showed a marginal significant decrease in the treatment group (6.69 ± 7.72, 5 [2–8]) as compared with the control group (13.81 ± 14.66, 6 [2–25]) ($p = 0.092$).

Discussion

In this clinical trial, we evaluated the role of vitamin D3 supplementation in preventing the conversion of ON to MS in patients with low serum 25 (OH) D statuses. We selected patients presenting with ON as this is a type of CIS that carries a particularly high risk of MS conversion. Our results indicate that vitamin D3 protects ON patients from developing MS.

The immunomodulatory effects of vitamin D3 on MS, which mediate a shift to a more anti-inflammatory immune response, especially by the promotion of regulatory T cell function, have been confirmed in several studies [12]. A decrease in inflammatory cytokines associated with MS symptoms (IL-2, TNF-α and IFN-γ), and an increase in anti-inflammatory cytokines (TGF-β, IL-10), have been verified to occur when the active form of vitamin D (1,25 (OH) D3) is administered [6, 13].

The main goals in the treatment of CIS patients are delaying the commencement of subsequent demyelinative-inflammative relapses, decreasing the progression of MRI lesions, and thereby postponing conversion to CDMS [14].

The use of calcitriol or ultraviolet radiation before experimental autoimmune encephalomyelitis induction (EAE, an animal model of MS and CNS inflammation) has been found to inhibit the commencement of the disease. Additionally, the administration of vitamin D3 at the onset of EAE prevents its progression [6, 15].

The association between serum 25 (OH) D levels and the risk of MS onset and the beneficial effects of vitamin D3 supplementation on the course of established MS have been indicated in several studies [15, 16]. It has been reported that women who take >400 IU/day vitamin D3 supplementation have less chance of developing MS than those who do not [17]. Higher 25 (OH) D concentrations

Table 1 The mean ± SD; and, median [range] and incidence rate-ratio of demyelinating plaques in MRI of patients at the time of ON diagnosis and after 1-year follow-up

Group	BH1[a]	BH2[b]	GE1[c]	New GE[d]	C1[e]	C2[f]	PV1[g]	PV2[h]	JC1[i]
Control group	1.36 ± 2.20;	1.64 ± 2.11;	1 ± 1.47;	1.36 ± 2.24;	3.36 ± 4.61;	3.36 ± 4.29;	5.18 ± 5.26;	5.91 ± 6.12;	3 ± 3.71;
	0 [0–6]	1 [0–6]	0 [0–4]	0 [0–6]	1 [0–13]	1 [0–6]	3 [0–16]	4 [1–18]	1 [0–9]
Treatment group	0.54 ± 1.45;	0.15 ± 0.55;	0.15 ± 0.36;	0 ± 0;	1.23 ± 2.74;	1.08 ± 3;	5 ± 4.9;	4.62 ± 4.29;	1 ± 1;
	0 [0–5]	0 [0–2]	0 [0–1]	0 [0]	0 [0–13]	0 [0]	3 [0–16]	3 [1–16]	1 [0–3]
Incidence rate ratio (95 % CI)	–	0.16 [0.037–0.742]	–	0.04 [0–0.236]	–	0.32 [0.173–0.592]	–	0.78 [0.549–1.109]	–
P value*	–	0.001	–	0.002	–	0.001	–	0.16	–

Group	JC2[j]	CC1[k]	CC2[l]	BS1[m]	BS2[n]	CR1[o]	CR2[p]	New T2[q]
Control group	2.82 ± 3.94;	1 ± 1.48;	1.27 ± 1.90;	0.36 ± 0.50;	0.27 ± 0.46;	0.18 ± 0.40;	0.18 ± 0.40;	2.63 ± 3.31;
	1 [0–13]	0 [0–4]	0 [0–5]	0 [0–1]	0 [0–1]	0 [0–1]	0 [0–1]	1 [0–10]
Treatment group	0.69 ± 1.10;	0.15 ± 0.55;	0.15 ± 0.55;	0.15 ± 0.55;	0.15 ± 0.55;	0 ± 00;	0 ± 00;	0.64 ± 0.65;
	0 [0–3]	0 [0–2]	0 [0–2]	0 [0–2]	0 [0–2]	0 [0]	0 [0]	0 [0–2]
Incidence rate ratio (95 % CI)	0.25 [0.117–0.516]	–	0.12 [0.027–0.532]	–	0.54 [0.047–4.92]	–	Not computable	0.17 [0.073–0.422]
P value*	0.001	–	0.005	–	0.84	–	–	0.001

* *P* values relate to between group comparisons of incidence rates in the final stage

[a] Black hole(s) in the first MRI

[b] Black hole(s) in the final MRI

[c] Gadolinium-enhancing lesion(s) in the first MRI

[d] New gadolinium-enhancing lesion(s) in the final MRI

[e] Cortical plaque(s) in first MRI

[f] Cortical plaque(s) in final MRI

[g] Periventricular plaque(s) in the first MRI

[h] Periventricular plaque(s) in the final MRI

[i] Juxtacortical plaque(s) in the first MRI

[j] Juxtacortical plaque(s) in the final MRI

[k] Corpus callosum plaque(s) in first MRI

[l] Corpus callosum plaque(s) in the final MRI

[m] Brain stem plaque(s) in the first MRI

[n] Brain stem plaque(s) in final MRI

[o] Cerebellum plaque(s) in the first MRI

[p] Cerebellum plaque(s) in the final MRI

[q] New plaque(s) in T2-weighted

(99.1–152.9 nmol/L) in comparison with lower levels, (25 (OH) D <63.3 nmol/L), have been associated with a decreased risk of MS in white patients [18].

These findings, in line with our results, support the importance of vitamin 25 (OH) D levels in the prevention of MS. The conversion of ON to MS in the treatment group was significantly lower than that in the control group (RR = 0.316, $p = 0.007$). The incidence rate of cortical, juxtacortical, corpus callosal and more importantly, new gadolinium-enhanced, new T2 lesions, and T1-weighted black holes, was significantly lower in the treatment group, after serum 25 (OH) D optimization (Table 1), than in the control group. However, the mean number of total MRI-detected plaques in the treatment group showed a marginal significant decrease in comparison with the control group. This is probably due to slight differences in the incidence rates of plaques at other sites of the brain (periventricular, brainstem, and cerebellum plaques). These findings indicate the role of vitamin D3 in preventing the development of demyelinating MRI lesions. There is a similar report concerning the decrease of gadolinium-enhancing lesions through the administration of vitamin D3 in the active phase of MS ($p = 0.03$) [7].

In addition, the safety of vitamin D3 supplements, even in doses higher than 2,000 IU/day, (the tolerable upper intake level, UL), has been reported in many studies [6]. However, the level of serum 25 (OH) D needed to guarantee the occurrence of the immunomodulatory effects, which can alter the demyelination/inflammation course has not yet been clearly specified [19].

As far as we are aware, the presence of MRI lesions in CIS patients is the most accurate predictor of the future outcome of their first demyelinative episode. Thus, according to our clinical and radiological results, the administration of supplements would be beneficial to optimize serum 25 (OH) D status in ON patients with vitamin D deficiency or insufficiency. Aside from the novelty of the subject of this pilot clinical trial, its results should be interpreted cautiously for it concerns a low number of patients who were traced for only 12 months. Extension of the size of study groups and further follow-up duration are needed to better clarify the value of vitamin D3 supplements in this regard.

Conclusion

The immunomodulatory effects of vitamin D3 can delay or prevent future relapses in CIS patients, even after the first demyelinative attack, thus reducing the risk of MS development. Low serum 25 (OH) D concentrations accelerate the pathologic pathways that result in MS development. We recommend the optimization of serum 25 (OH) D status in all patients presenting with ON who have low serum levels of this vitamin. We also propose that all other CIS patients who are at risk of developing MS should be similarly treated. This is an inexpensive and safe intervention [9, 20]. Other larger investigations with a longer follow-up period are needed to confirm our results.

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Conflict of interest The authors have no proprietary interest in the materials presented herein.

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