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Research Article

Influence of High Doses Vitamin D Therapy at Immunological Parameters in Multiple Sclerosis Patients. A Systematic Review

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Abstract

Multiple sclerosis (MS) is a chronic autoimmune disease of central nervous system which etiology remains unclear. Worldwide studies suggest strong link between vitamin D deficiency and MS. In this study, we conducted a systematic literature search regarding the effect of high dose vitamin D treatment in immunological parameters of MS patients. High dose vitamin D treatment promotes few significant chances in immunological systemic markers, mainly in the total amount of peripheral blood IL-17+CD4+ T cells and in the proportion of TH IL-4+ cells. In summary, high dose treatment promotes limited differences in immunological markers in patients with MS.

Keywords: Multiple Sclerosis; Vitamin D; Treatment; Systematic Review; Supplementation

Abbreviations

MS: Multiple sclerosis; CNS: Central Nervous System; VDR: Vitamin D Receptor; EAE: Experimental Autoimmune Encephalomyelitis; MEDLINE: Medlars Online International Literature; MeSH: Medical Subject Headings; IFN- β: Interferon-β; PBMC: Peripheral Blood Mononuclear Cells; LAP: Latency Activated Protein; QOL: Quality of Life; RRMS: Relapsing-Remitting Multiple Sclerosis; EDSS: Expanded Disability Status Scale; MRI: Magnetic Resonance Imaging; ARR: Annualized Relapse Rate; VLA-4: Very late antigen-4; CSF: Cerebrospinal Fluid; RCTs: Randomized Clinical Trials

Introduction

Multiple sclerosis (MS) is a chronic, degenerative and demyelinating disease of the central nervous system (CNS). The main mechanism of MS seems to be an autoimmune inflammatory reaction which attacks and destroys the myelin sheath, resulting in a gradual exacerbation of neurological dysfunction [1].

Clinical features of MS, in most cases, are identified by outbreaks (relapses, acute exacerbations) and vary according to the affected site [2]. Dysfunction originating from hemispherical motor/sensory, cerebellar, spinal cord and optic nerves are common [3].

There are approximately 2.5 million individuals affected by MS worldwide [3,4]. The etiology of MS remains unclear but, it is generally assumed that MS susceptibility is affected by genes and environmental factors such as Epstein-Barr viral infection, smoking and vitamin D levels [5-7]. Worldwide studies have suggested a strong link between vitamin D deficiency and MS [8].

Vitamin D is a type of fat-soluble secosteroid which can be synthesized in the skin in response to sun exposure [9]. The potential role of vitamin D and its bioactive metabolite ($1,25(OH)_2D_3$) in immunomodulatory responses are mediated by the vitamin D receptor (VDR) [10]. VDR is expressed in immune cells, such as macro-

phages, dendritic cells, and activated T and B lymphocytes, and it is involved in proliferation and maintenance of B-cell homeostasis, inhibition of dendritic and T-helper cell (CD4+) differentiation, enhancement of T regulatory cell proliferation and suppression of inflammatory mediator release [10-12].

Since vitamin D has an immunoregulatory property, several studies analyzed the effects of vitamin D on immune response [13-15]. In experimental autoimmune encephalomyelitis (EAE), therapeutic use of vitamin D or $1,25(OH)_2D_3$ showed positive results. According to Chang., *et al*. [16], $1,25(OH)_2D_3$ reduced the migration and differentiation of T-helper 17 cells in EAE. Furthermore, Chiuso-Minicucci., *et al*. [17] reported a significant decrease in number of mature splenic dendritic cells after administration of $1,25(OH)_2D_3$.

Based on preclinical studies, several clinical investigations were performed evaluating the effect of vitamin D treatment in human with MS, and recently, several studies reported the effect of high dose therapy and its immunomodulatory effects in promoting an anti-inflammatory state [15,18,19].

Given the potential immunomodulatory effects of high dose vitamin D therapy in MS, the aim of this study was to compile data about the effect of high dose vitamin D treatment in immunological parameters of MS patients.

Materials and Methods

Study registration

This systematic review was registered at PROSPERO database (International prospective register of systematic reviews) with ID: CRD42018088314.

Search strategy

A systematic literature search was performed in May 2019 for clinical trials regarding the relationship between MS and vitamin D. The database used were Cochrane, Medlars Online International Literature (MEDLINE) via PubMed, EMBASE and ClinicalTrials.gov. Medical Subject Headings (MeSH) dictionary was used to identify the keywords used in the search. The terms used were "multiple sclerosis" and "vitamin D". We searched for publications between 2013 and 2019 and limited to clinical trials in humans.

Study selection

Two reviewers (IMRFRM and AMV) screened the articles independently by assessing titles and abstracts. Articles not fitting the eligibility criteria and duplicate articles were excluded. The full text of the remaining articles was read to assess for inclusion.

Inclusion and exclusion criteria

Inclusion criteria was studies with patients diagnosed with MS undergoing conventional treatment with interferon-β (IFN- β) or not; receiving high dose vitamin D as an intervention; low dose or placebo as comparator. Studies that did not present immunological parameters after high dose vitamin D supplementation and those scored 2 or less by Jadad classification system were excluded.

Data extraction

To minimize selection bias, two reviewers independently screened the search findings (title/abstracts). After screening title and abstracts, all texts not presenting immunological parameters were discarded. Disagreements between the two reviewers were resolved by consultation with a third reviewer.

Quality assessment

To assess the quality of selected studies, Jadad classification system was used [20]. Selected studies were scored according to the presence of key methodological features as follows: 1 point were awarded, for each item, for the presence of the description of randomized, appropriate method of randomization, described as double blind, appropriate method of double blinding and the description of withdrawals and dropouts; 1 point was deducted, for each item, if the method to generate randomization and blinding was inappropriate.

Results

Search results

The search initially identified 100, 27, 189 and 3 articles at Cochrane, PubMed, EMBASE and ClinicalTrials.gov databases, respectively. After duplicated articles exclusion, 219 references remained. Thirty-seven articles remained after titles and abstracts screening by the two reviewers. Among these studies, 9 were excluded due to lack of full texts, 4 were missing results, 12 did not discuss immunological parameters and 1 was excluded for exclusion criteria. After this initial selection, 11 studies remained and they were submitted to assess the quality of the clinical trial using the Jadad score (Supplementary table 1) [18,21-30]. Studies with score 2 or less were excluded by the low evidence supported by the quality. At the end, 8 studies were selected for qualitative synthesis (Figure 1).

Jadad Score Calculation											
Item	**Article**										
	Golan D., *et al.* 2013	**Aivo, J., *et al.* 2015 ***	**Ashtari F., *et al.* 2015**	**Farsani ZS., *et al.* 2015**	**Gargari BN., *et al.* 2015**	**Røsjø E., *et al.* 2015**	**Toghianifar N., *et al.* 2015**	**Muris AH., *et al.* 2016 ***	**Sotirchos, ES., *et al.* 2016**	**Rolf, L., *et al.* 2018 ***	**Rolf, L., *et al.* 2019 ***
Was the study described as randomized (this includes words such as randomly, random, and randomization?)	1	1	1	1	0	1	1	1	1	1	1
Was the method used to generate the sequence of randomization described and appropriate (table of random numbers, computer-generated, etc.?)	0	1	0	0	0	0	0	1	1	1	1
Was the study described as double blind?	1	1	1	0	0	1	1	1	1	1	1
Was the method of double blinding described and appropriate (identical placebo, active placebo, dummy, etc.)?	0	1	0	0	0	0	0	0	1	0	0
Was there a description of withdrawals and dropouts?	1	1	0	0	0	1	1	1	1	0	0
Deduct one point if the method used to generate the sequence of randomization was described and it was inappropriate (patients were allocated alternately, or according to date of birth, hospital number, etc.)	0	0	0	0	0	0	0	0	0	0	0
Deduct one point if the study was described as double blind but the method of blinding was inappropriate (e.g. comparison of tablet vs. injection with no double dummy).	0	0	0	0	0	0	0	0	0	0	0
Total	3	5	2	1	0	3	3	4	5	3	3

Supplementary Table 1: Jadad classification system. Selected studies were scored according to the presence of key methodological features as follows: 1 point were awarded, for each item, for the presence of the description of randomized, appropriate method of randomization, described as double blind, appropriate method of double blinding and the description of withdrawals and dropouts; 1 point was deducted, for each item, if the method to generate randomization and blinding was inappropriate.

*: The randomization data were obtained from parental studies.

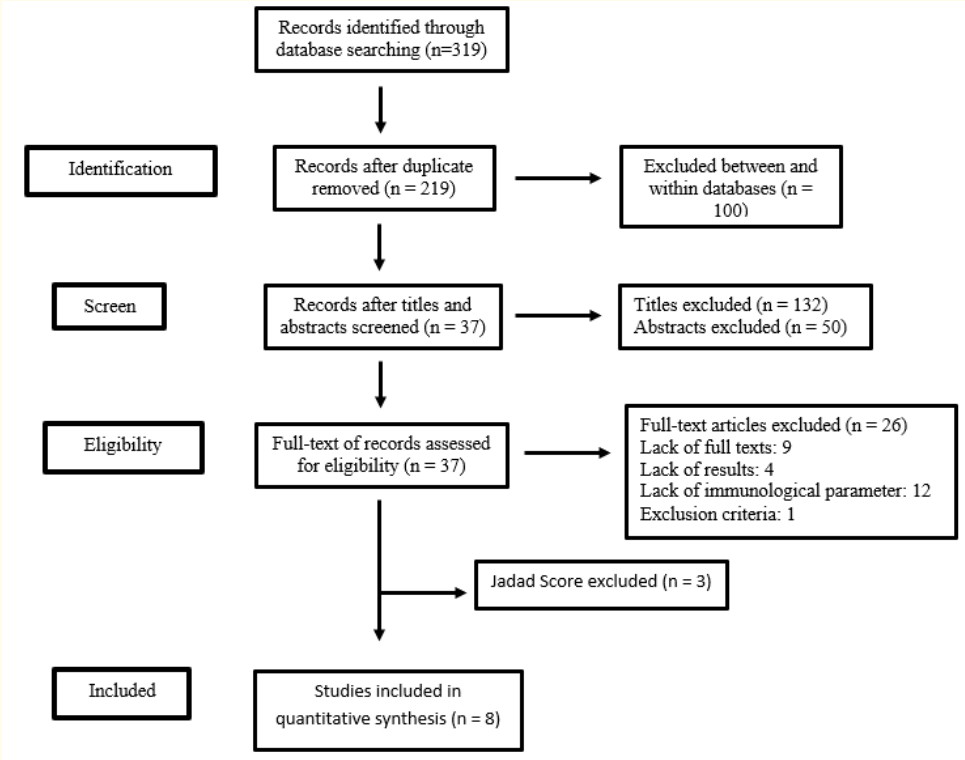

Figure 1: Literature search and review flow-chart for selection of studies.

Description of studies

From 8 included studies, 1 from Israel [24], 1 from Finland [21], 1 was from Norway [28], 1 from Iran [30], 1 from Netherlands [25], 1 from US [29] and 2 from Netherlands [26,27]. Table 1 and 2 summarizes all the data collected from selected studies including, vitamin D status and immunological parameters. After analyzes of selected studies quality (Supplementary table 1), 2 gained the maximum score [21,29], 1 scored 4 points and the others scored 3 points due lacking randomizing or method of double binding description [24-28,30].

Vitamin D supplementation

Data collected from analyzed articles (Table 1 and 2) represented 431 patients (222 individuals at high dose vitamin D treatment and 209 at conventional treatment). The treatment period varies from 12 to 96 weeks and vitamin D supplementation varies from 4,370 IU/day to 50,000 IU/5 days. Treatment resulted in increased level of serum vitamin D that reaches statistical significance in all analyzed articles. It is worth noting that the methods and dosage applied in the different studies are quite diverse, which do not contribute to reproducibility nor direct comparison among results.

Effects of vitamin D in immunological parameters

Data extracted from 5 clinical trials (Table 1) showed that there were no differences in serum levels of chemokines (CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CCL21, CXCL1, CXCL5, CXCL8, CXCL9, CXCL10 and CXCL16), adhesion molecules (ALCAM, ICAM-1 and VCAM-1), matrix degrading protein (MMP-9), inflammatory markers (OPG, OPN, PTX3, sFRP3, Leptina, CM-CSF, M-CSF, G-CSF, Resistin, PAI1, FGFb, PDGFbb, sFASL, sCD40L, NGF, VEGF, TNF-α, TNF-β, IFN-β, IFN-γ, IL-1A, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-13, IL-15, IL-12p40, IL-12p70, IL-17A, IL-17F, IL-22, LIF, HGF and SCF), anti-inflammatory markers (TGF-β1, LAP and IL-10) and soluble receptors (IL-1Ra, sTNF-R1) [24,28-30] among the different treatment groups. When low dose group was analyzed, Golan., *et al.* [24] reported an elevated serum level of IL-17 after 12 weeks of vitamin D treatment. Also, Åivo., *et al.* [21] showed a significant increase in circulating levels of LAP-1 only in vitamin D treatment group.

Authors, year	Number of patients at baseline (control/treatment)	Research period	Intervention		Dosage of vitamin d (baseline / end-study)			Immunological parameters (baseline / end-study)			
			Control	Treatment group	Control	Treatment group	P value Vitamin d vs control	Marker	Control	Treatment group	P value (treatment vs control)
Golan D., *et al.* 2013	21/21	12 weeks	IFNβ + Bottle containing placebo + 800 IU/ day	IFNβ + Bottle containing 75,000 IU/ every 3week + 800 IU daily tablets (total 4370 IU/day)	48 ± 14.2 to 68 ± 11nmol/L	48.2 ± 13.9 to 122.6 ± 32 nmol/L	<0.001	IFN-γ (pg/mL) IL-17 (pg/mL) IL-10 (pg/mL)	0.20 ± 0.22 / 0.14 ± 0.2 4.01 ± 3.99 / 9.14 ± 9.9 undetected	0.51 ± 1.1 / 0.58 ± 1.3 5.83 ± 6.1 / 6.38 ± 6.7 undetected	**1-** 0.13 **2-** 0.7 **3-** 0.04 **4-** 0.75 -
Aivo J., *et al.* 2015	29/30	48 weeks	IFNB-1b and placebo capsules (SwissCaps, Switzerland)	IFNB-1b and Cholecalciferol (Dekristol) containing 20 mg of cholecalciferol, corresponding to 20 000 IU or 0.5 mg of vitamin D3, in arachis oil inside a refined gelatine capsule for once weekly.	17 – 94 nmol/l	67 – 163 nmol/l	p<0.0001	LAP (pg/mL) IFN-g (pg/mL) IL-17A (pg/mL) IL-2 (pg/mL) IL-10 (pg/mL) IL-9 (pg/mL) IL-22 (pg/mL) IL-6 (pg/mL) IL-13 (pg/mL) IL-4 (pg/mL) IL-5 (pg/mL) IL-1b (pg/mL) TNF-a (pg/mL)	39.1 to 49.5 332.0 to 334.8 307.2 to 329.8 164.5 to 155.9 34.8 to 22.6 187.9 to 181.7 464.7 to 449.4 9.1 to 7.00 111.9 to 133.4 52.6 to 61.4 139.0 to 113.4 83.4 to 111.8 13.6 to 18.1	46.6 to 54.8 565.9 to 767.0 326.8 to 387.5 239.2 to 288.5 25.1 to 43.6 117.0 to 133.3 521.2 to 498.8 9.7 to 11.4 166.2 to 173.3 92.0 to 100.9 147.0 to 183.5 106.6 to 120.5 22.9 to 29.2	**5-** 0.0249 - - - - - - - - - - - -
Røsjø E., *et al.* 2015	32/36	96 weeks	Immunomodulatory treatment (i.e., IFN-β, glatiramer acetate, or natalizumab) + placebo	Immunomodulatory treatment (i.e., IFN-β, glatiramer acetate, or natalizumab) + 20,000 IU Vitamin D (DekristolTM, Mibe GmbH Arzneimittel, Brehna, Germany)	57.0 to 63.1 nmol/L	55.6 to 123.2 nmol/L	<0.001		Change (SD)	Change (SD)	P adjusted
								ALCAM[a] (ng/mL)	9 (22)	4 (31)	0.441
								CCL21[b] (pg/mL)	30 (115)	66 (129)	0.116
								CXCL16[c](pg/mL)	47 (242)	42 (224)	0.952
								IL-1Ra[d] (pg/mL)	- 10 (115)	98 (478)	0.487
								MMP-9[e] (ng/mL)	84 (529)	170 (464)	0.306
								OPG[f] (pg/mL)	- 33 (228)	43 (307)	0.246
								OPN[g](ng/mL)	- 2.0 (2.5)	- 1.4 (3.1)	0.621
								PTX3[h] (pg/mL)	- 95 (458)	- 39 (566)	0.912
								sFRP3[i] (pg/mL)	129 (736)	13 (3775)	0.548
								sTNF-R1[j] (pg/mL)	83 (139)	107 (164)	0.924
								TGF-β1[k] (ng/mL)	0.1 (2.2)	0.0 (2.9)	0.971

Toghianifar N., *et al*. 2015	45/44	12 weeks	IFNβ + placebo	IFNβ + 50,000 IU (vitamin D3 Pearl, 50,000 IU, Zahravi pharmaceutical Co., Tabriz, Iran) every five days	39.6 ± 20.97 to 28.66 ± 25.34 ng/ml	28.27 ± 29.03 to 84.67 ± 42.87 ng/ml	<0.001	IL-17 (pg/mL)	30.31 ± 75.85 to 46.13 ± 94.70	56.75 ± 28.72 to 58.93 ± 67.93	0.960
Sotirchos ES., *et al*. 2016	21/19	24 weeks	Immunomodulatory treatment (i.e., IFN-β, glatiramer acetate, natalizumab, fingolimod or abatacept) + 400 IU/day cholecalciferol (Continental Vitamin Company, Vernon, CA) + 400 IU from daily multivitamin (LuckyVitamin, Conshohocken, PA)	Immunomodulatory treatment (i.e., IFN-β, glatiramer acetate, natalizumab, fingolimod or abatacept) + 10.000IU/day cholecalciferol (Continental Vitamin Company, Vernon, CA) + 400 IU from daily multivitamin (LuckyVitamin, Conshohocken, PA)	27,9 to 34,8 ng/mL	27,1 to 62 ng/mL	<0.0001	Fifty-one cytokines [l]	-	-	-

Table 1: Descriptive analysis of circulating cytokine levels in the reviewed studies.

[a]Activated leukocyte cell adhesion molecule; [b]Chemokine (C–C motif) ligand 21; [c]Chemokine (C–X–C motif) ligand 16; [d]Interleukin-1 receptor antagonista; [e]Matrix metalloproteinase 9; [f]Osteoprotegerin; [g] Osteopontin; [h]Pentraxin 3; [i]Secreted frizzled-related protein 3; [j]Soluble tumor necrosis factor receptor 1; [k]Transforming growth factor beta 1.

1- IFN-γ (low dose group); 2- IFN-γ (high dose group); 3- IL-17 (low dose group); 4- IL-17 (high dose group).

5- Only on vitamin D group.

[l] Leptin, ENA78 (CXCL5), IL-17F, IFN-b, Groa (CXCL1), GM-CSF, IL-13, TNF-α, LIF, IL-1β, VEGF, IL-5, IL-10, MCP-1(CCL2), IFN-g, IL-12p70, IL-7, IL-2, MCP3 (CCL7), IL-15, IL-4, FGFb, IL-6, IP-10 (CXCL10), PDGFbb, TGF-β, HGF, IL-12p40, IL-1A, SCF, TGF-A, NGF, Mig (CXCL9), M-CSF, TNF-β, sFASL, IFNA, Mip1a (CCL3), IL-1RA, TRAIL, IL-17, Mip1β (CCL4), G-CSF, Resistin, ICAM-1, Eotaxin (CCL11), IL-8, VCAM-1, CD40L, RANTES (CCL5), PAI1.

Authors, year	Number of patients at base line (control/ treatment)	Research period	Intervention		Dosage of vitamin d (baseline/end-study)			Immunological parameters (baseline/end-study)			
			Control	Treatment group	Control	Treatment group	P value Vitamin d vs control	Marker	Control	Treatment group	P value (treatment vs control)
Sotirchos ES., *et al.* 2016	21/19	24 weeks	Immunomodulatory treatment (i.e. IFN-β, glatiramer acetate, natalizumab, fingolimod or abatacept) + 400 IU/day cholecalciferol (Continental Vitamin Company, Vernon, CA) + 400 IU from daily multivitamin (LuckyVitamin, Conshohocken, PA)	Immunomodulatory treatment (i.e. IFN-β, glatiramer acetate, natalizumab, fingolimod or abatacept) + 10.000 IU/day cholecalciferol (Continental Vitamin Company, Vernon, CA) + 400 IU from daily multivitamin (LuckyVitamin, Conshohocken, PA)	27,9 to 34,8 ng/mL	27,1 to 62 ng/mL	<0.0001	IL-17^{+}CD4^{+} T cells IFN-g^{+}CD4^{+} T cells IFN-g^{+}IL-17^{+} CD4^{+} T cells Effector memory CD4^{+} T cells Central memory CD4^{+} T cells Naive CD4^{+} T cells CD161^{+}CD4^{+} T cells CD85j^{+} CD8^{+} T cells	6.57 ± 5.16/6.13 ± 5.64 29.93 ± 17.16/27.66 ± 12.86 2.94 ± 2.72/2.79 ± 3.52 38.51 ± 18.4/37.71 ± 17.19 53.84 ± 14.88/55.29 ± 15.88 34.18 ± 12.87/36.0 ± 12.44 6.11 ± 3.27/5.70 ± 3.45 15.54 ± 13.99/15.0 ± 13.45	9.32 ± 7.97/5.62 ± 6.04 29.77 ± 15.49/22.27 ± 13.13 3.73 ± 4.24/2.03 ± 2.91 40.56 ± 20.56/30.69 ± 19.71 50.07 ± 21.88/60.96 ± 24.43 38.94 ± 11.96/42.2 ± 13.49 5.51 ± 2.66/4.7 ± 3.01 15.87 ± 19.06/12.68 ± 15.13	0.039 0.20 0.20 0.12 0.16 0.33 0.37 0.066
Muris AH., *et al.* 2016	23/30	48 weeks	IFNβ-1a (Rebif®, Merck Serono S.A., Darmstadt, Germany) + placebo	IFNβ-1a and vitamin D_3 7000 IU/day (for 4 weeks, cholecalciferol, Vigantol® Oil, Merck KGaA, Darmstadt, Germany) followed by 14000 IU/day to the end.	36 - 85 nmol/L	162 - 250 nmol/L	p<0.001	Regulatory lymphocytes [a] % IL-4^{+} THcells [c] LAP (pg/mL) [c] Cytokine production after PBMC stimulation [b]	- 3.7 to 2.9 410.2 - 674.0 to 689.1 - 1097.7 -	- 4.1 to 4.1 527.0 - 907.3 to 549.8 - 942.2 -	- 0.05 <0.01 -
Rolf L., *et al.* 2018	23/30	48 weeks	IFNβ-1a (Rebif®, Merck Serono S.A., Darmstadt, Germany) + placebo	IFNβ-1a and vitamin D_3 7000 IU/day (for 4 weeks) (cholecalciferol, Vigantol® Oil, Merck KGaA, Darmstadt, Germany) followed by 14000 IU/day to the end.				IL-12RA m RNA levels from PBMC CD 25 on: Total CD3+CD4+ Conventional T cells CD3+CD4+CD25+ FOXP3+ T reg CD3+CD4+CD25+ CD127- Treg CD3+CD4+CD25+ CD127-Foxp3+ Treg	0.21 - 1.96 650 - 1071 to 596 - 1087 605 - 961 to 551 - 992 4257 - 5475 to 3369 - 4992 4559 - 6172 to 3346 - 5721 4829 - 6418 to 3801 - 5935	0.35 - 2.19 721 - 1082 to 692 - 1065 648 - 994 to 648 - 994 4371 - 5521 to 3928 - 5152 4687 - 6302 to 4307 - 5461 4996 - 6235 to 4822 - 5809	0.895 0.744 0.788 0.106 0.136 0.136

Rolf L., *et al*. 2019	15/13	16 weeks	Treatment with injectable or oral disease modifying drugs (DMD; interferon-beta, glatiramer acetate, dimethyl fumarate, teriflunomide or fingolimod) plus matched placebo.	Treatment with injectable or oral disease modifying drugs (DMD; interferon-beta, glatiramer acetate, dimethyl fumarate, teriflunomide or fingolimod) plus 4000 IU/day vitamin D3 drops (Vigantol Oil, Merck, Darmstadt, Germany),	71 - 95 to 61 - 90 nmol/L	71 - 132 to 123 - 144 nmol/L	P0.005	IFN-γ [d] IL-4 IL-10 IL-17A TNF-a GM-CSF	10.5 - 23.6 to 9.1 - 20.1 3.6 - 8.2 to 3.9 - 8.6 0.4 - 1.0 to 0.3 - 1.1 1.1 - 2.4 to 1.2 - 2.7 24.9 - 54.8 to 27.6 - 52.4 9.7 - 24.8 to 9.1 - 22.7	10.2 - 24.0 to 6.6 - 20.6 4.1 7.8 to 4.1 - 6.3 0.1 - 1.1 to 0.2 - 0.6 0.8 - 2.6 to 0.6 - 2.3 25.3 - 53.2 to 28.6 - 51.5 7.5 - 23.0 to 7.2 - 20.1	0.236 0.981 0.443 0.456 0.829 0.548

Table 2: Descriptive analysis of culture stimulated cells in the reviewed studies.

[a]Proportion of regulatory lymphocytes: CD25+CD127- nTreg; CD25+FoxP3+ nTreg; CD25+CD127-FoxP3+ nTreg; CD39+ nTreg; memory nTreg; iTreg; Breg

[b]Proportion of cytokine producing T helper cells in placebo or vitamin D3 supplemented IFNβ treated RRMS patients. Cytokines analyzed: IL-4, IFN-γ, IL-17, IL-22, GM-CSF, TNF-α.

[c]Anti CD3 stimulated PBMB - cytokines analyzed: IL-10, IL-4, IL-5, IFN-γ, IL-17, IL-22, GM-CSF, TNF-α.

[d]Intracellular staining of CD3+CD8− T cells after 72 h stimulation with anti-CD3, followed by re-stimulation with PMA/ionomycin.

In contrast, at cellular level, there is no difference in several T and B cell population after high dose treatment (Table 2). No significant differences were observed between the two groups in proportion of IFN-γ+CD4+ T, IFN-γ+IL-17+ CD4+ T, Effector memory CD4+ T, Central memory CD4+ T, Naive CD4+ T, CD161+CD4+ T, CD85j+ CD8+ T cells, CD25+CD127- nTreg, CD25+ Foxp3+ nTreg, CD25+ CD127-Foxp3+ nTreg, CD39+ nTreg, Memory nTreg, iTreg and B reg cells [25,29]. In addition, there is no difference in cytokine production by T cell, such as IFN-γ, IL-4, IL-10, IL-17A, IL-22, GM-CSF and TNF-α [25,26].

However, some differences are present when groups are analyzed separated. When high dose group were analyzed, at baseline and at the end of the study, there was a significant decrease in circulating CD161+CD4+ T cells, Effector memory CD4+ T cells, CD85j+ CD8+ T cells and a significant increase in Central memory CD4+ T and CD45RA+ CD4+ cells [29]. In another study, it was shown that there is a decreased percentage of IL-4+TH cells in the control group and a maintenance of this population in the high dose group, and after stimulation of peripheral blood mononuclear cells (PBMC), it was observed an increase in the proportion of latency activated protein (LAP) of TGF-β and IL-5 positive cell in control group [25].

A contradictory data is observed in the proportion of IL-17+ cells. Sotirchos., *et al*. [29] showed that high dose vitamin D supplementation, promoted a significant reduction in the proportion of IL-17+CD4+ T cells when compared with low dose treatment group. In contrast, Muris., *et al*. [25] found no difference in IL-17+ TH cells between the groups. Finally, Rolf., *et al*. [27] showed that after vitamin D supplementation, there was no difference on IL-12RA mRNA expression in PBMC from control and vitamin D treatment group. In addition, there was no difference in expression of CD25 protein in total CD3+CD4+, conventional T cells, CD3+CD4+CD25+FOXP3+ Treg, CD3+CD4+CD25+CD127- Treg and CD3+CD4+CD25+CD127-FOXP3- Treg cells. However, there was a significant decrease in levels of soluble CD25 in the placebo group.

Discussion

In this systematic review, we summarized clinical data of the influence of high dose vitamin D supplementation on immunological components in patients with multiple sclerosis by search randomized, placebo-controlled, double-blind trials published from 2013 to 2018 in PubMed/Medline, Cochrane Central Register of Controlled Trials, Embase and Clinical Trails.com databases. The results of this present systematic review led to some conclusions: First, a bias to be considered is the lack of dose standardization and duration of treatment. Second, there were no changes in soluble circulating immunological parameters after high dose supplementation. Third, high dose treatment altered the phenotype of circulating immunological cells, with reduction in the proportion of IL-17+CD4+ T cells in high dose treatment group when compared to low dose or conventional treatment group.

Several clinical trials and observational studies have shown the importance of high-dose vitamin D supplementation for MS treatment. Ashtari., *et al*. [18] compared Quality of Life (QOL) in Relapsing-Remitting Multiple Sclerosis (RRMS) patients receiving high dose vitamin D for 3 months with placebo group. Mental QOL showed significant improvement in vitamin D group after adjusting for sociodemographic and Expanded Disability Status Scale (EDSS) scores. Furthermore, according to Fitzgerald., *et al*. [31], 25(OH)D was significantly inversely correlated with the cumulative number of active lesions between baseline and the last Magnetic Resonance Imaging (MRI) (average follow-up time, 2 years), with a 50-nmol/L higher level in serum 25(OH)D levels associated with a 31% lower rate of new lesions. However, recently meta-analysis pointed contradictory results. Zheng., *et al*. [32] showed that vitamin D3 treatment did not promote therapeutic effect on MS according to EDSS score and Annualized Relapse Rate (ARR). Contradictory results have been pointed in several works and may be due differences in dose and frequency of vitamin D intake and must be considered when evaluating the impact on immune response [30,33,34].

Classically, MS is regarded as a T cell-mediated autoimmune disorder with the presence of T-cell subsets at lesions, leading to activation of the entire inflammatory phenotype [35]. High numbers of T cells expressing the transcription factor T-bet is suggestive of the presence of T cells polarized into a γ-interferon-producing subset [36]. However, our analyses showed that, after high dose vitamin D supplementation, there was no decrease in the total amount of serum IFN-γ and the percentage of circulating IFN-γ T cells [24,25,37].

Another cell type present is CD4+ IL-17+ T cell (TH17 cells), pointed as a major contributor to the immunopathogenesis of MS [29]. The meta-analysis of Li., *et al*. [38] showed that MS patients present a higher proportion of TH17 cells in peripheral blood and elevated IL-17 and IL-23 serum levels when compared to controls. IL-17 is the hallmark cytokine produced by TH17 cells, involved in the process of demyelination, infiltration of granulocytes, activation of microglial cells, and in the induction of chemokines production, including CCL2, CCL5, and CCL20 [39,40]. Our analyses showed no differences in serum levels of IL-17 after high dose vitamin D supplementation. However, there was an increase in serum concentration of IL-17 in MS patients submitted to a low dose treatment [24]. At the cellular level, according to Sotirchos., *et al*. [29] there was a decreased in TCD4+IL-17+ cells after high dose treatment when compared to low dose group. In addition, there was a decrease in CD161+CD4+ T cells, a marker of pathogenic TH17 cells [41]. All of this data suggest that the main mechanism of vitamin D3 action in MS is by controlling the proportion and function of TH17 cells.

Another important result is the decrease in the percentage of circulating effector memory CD4+ T cells and an increase in circulating central memory CD4+ T cells and CD45RA+ CD4+ T cells in high dose group patients [29]. These findings are in accordance with a previous *in vitro* study where PBMC was treated with vitamin D, resulting in an increased proportion of circulating naïve CD4+ T cells and T central memory, and a decrease in T effector memory cells [42]. Thus, reduction in the number of effector memory T cells and increasing in the number of central memory T cells could be an immunomodulatory mechanism, since the first is related to migration to inflamed tissue to display effector functions, like cytokine production, and the second is associated to migration to secondary lymphoid tissue [43]. A possible explanation of this vitamin D effect resides in the fact that $1,25(OH)_2D_3$ negatively regulates the expression of very late antigen-4 (VLA-4) in human leukemic cells [44]. VLA-4 is an integrin that consists of CD49d and CD29 subunit, and it is involved in T cell migration into the CNS [45]. All of these results suggest that vitamin D promote a shift in the immunological cell phenotype that favors diminishing inflammatory T cells quantity at the lesion.

Dysfunction in regulatory mechanism present in MS pathogenesis is well documented. In MS patients, T Reg cells show lower suppressive capabilities, dysregulated production of IL-10, TGF-beta, and low sensitivity to IL-2 [37]. When we analyzed anti-inflammatory cytokines, we also observed that there were no differences in serum levels of IL-10 and TGF-β among groups [24,28].

Several inflammatory markers play a role in the pathogenesis of MS. According to Stelmasiak., *et al*. [46], IL-6 is elevated in serum and cerebrospinal fluid (CSF) of MS patients. MMP-9 is secreted by activated T cells and microglia, and it is involved in digestion of extracellular matrix and loss of blood brain barrier integrity and was positively associated with MRI activity in RRMS patients [47]. CXCL16 is expressed on the surface of antigen presenting cells acting as a ligand for CXCR6 on T cells and natural killer T cells. According to Holmøy., *et al*. [47], serum levels of CXCL16 reflects MRI

activity in RRMS, suggesting that this marker may predict disease. However, in our study, high dose treatment showed no immunomodulatory role on these mediators and among others fifty mediators. Probably, several factors may contribute to this finding, such as dose of vitamin D, extension of the treatment and half-life of the cytokines in circulation.

Furthermore, discrepant findings may be influenced by underpowered randomized clinical trials (RCTs) based on observational studies, under effect of confounding and reverse causality, which could impact in the detection of vitamin D supplementation effects. Genetic factors, sunlight exposure, metabolism, body mass index, also unknown factors in physical activity and diet may influence in vitamin D levels and in the disease course [48].

The first large RTCs, SOLAR [49] and CHOLINE [50], evaluated the effects of vitamin D3 (cholecalciferol) as add-on to IFN-β-1a in patients with RRMS. In SOLAR study, 229 randomized patients received oral high-dose of 14,007 IU/day of cholecalciferol or placebo for 48 weeks, whereas 129 randomized patients received 100,000 IU or placebo every other week for 96 weeks in CHOLINE study. Both studies did not reach their primary endpoints. SOLAR and CHOLINE could not establish beneficial effects for high-dose vitamin D3 as add-on to IFN-β-1a according to the proportion of patients with no evidence of disease activity in 48 weeks, and ARR in 96 weeks, respectively. However, their secondary endpoints results suggest moderate effects. In SOLAR, cholecalciferol supplementation appears to have a protective effect on the development of new MRI lesions, while the CHOLINE study showed reduced ARR, MRI parameters (less new hypointense T1 lesions and reduced volume of hypointense T1 lesions) and disability progression evaluated in 90 patients who completed the 2-year follow-up.

Although some evidence points to its beneficial effects, the role of vitamin D in MS is still in discussion. Recent studies have demonstrated that vitamin D serum levels do not relate to MS risk in non-Caucasian populations [51], and that higher levels of exposure to ultraviolet radiation from sunshine is consistently associated with a reduced risk for MS in different ethnicities [52], indicating that time in the sun may bring more health benefits than the vitamin D supplementation, especially in blacks and Hispanics.

Conclusion

Worldwide studies indicated a strong link between lower levels vitamin D and increased risk of MS, however, the impact of vitamin D supplementation on MS activity remains unclear.

This review analysis showed that after high dose vitamin D therapy there were few differences at the expression and production of immunological markers, as IL-17+CD4+ T cells. In addition, it was clear that the doses and duration of vitamin D supplementation were not standardized and this is probably the main reason of the divergent results.

Further studies are necessary to establish the recommended levels and duration of vitamin D supplementation to reduce the MS clinical activity. It is also necessary to clarify the molecular mechanisms of the immunomodulatory effects of vitamin D in patients with MS.

Conflict of Interest

The authors declare no conflicts of interest.

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