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Anti-inflammatory effects of dietary vitamin D_3 in patients with multiple sclerosis

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Neurol Genet 2018;4:e278. doi:10.1212/NXG.0000000000000278

Abstract

Objective

To assess the effects of dietary vitamin D_3 on proinflammatory (interleukin-17A [IL-17A] and IL-6) and anti-inflammatory (IL-10) cytokines.

Methods

Our study was conducted on 75 participants who were divided into 3 groups: multiple sclerosis participants (MSPs, n = 25), first-degree relative participants (FDRPs, n = 25), and healthy participants (HPs, n = 25). All groups received 50,000 IU vitamin D_3/wk for 8 weeks. Serum 25-(OH) vitamin D_3 levels and messenger RNA (mRNA) expression levels of ILs were determined using electrochemiluminescence assay and real-time PCR, respectively.

Results

Vitamin D_3 affected the levels of IL-17A, IL-10, and IL-6 among the 3 groups ($p < 0.001$ for all). Levels of IL-17A (MSPs: fold change [FC] = 5.9, $p = 0.014$; FDRPs: FC = 5.2, $p = 0.006$; HPs: FC = 4.2, $p = 0.012$) and IL-6 (MSPs: FC = 5.6, $p = 0.003$; FDRPs: FC = 5.5, $p = 0.002$; HPs: FC = 5.1, $p < 0.001$) were downregulated after vitamin D_3 treatment. In addition, levels of IL-10 (MSPs: FC = 6.2, $p = 0.005$; FDRPs: FC = 4.6, $p < 0.001$; HPs: FC = 5.2, $p < 0.001$) were upregulated after 8 weeks.

Conclusions

Although supplementation with vitamin D_3 reduced the mRNA expression levels of IL-17A and IL-6, it increased the mRNA expression level of IL-10 in all groups. However, these effects were more considerable in the MSP group than in the other groups. Of interest, in a deficiency state of serum vitamin D3, IL-17A expression had a positive feedback effect on the expression of IL-6. Conversely, in the sufficient state, IL-10 expression had a negative feedback effect on the expression of IL-17A and IL-6.

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Funding information and disclosures are provided at the end of the article. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

The Article Processing Charge was waived at the discretion of the Editor.

Glossary

APC = antigen-presenting cell; **BBB** = blood-brain barrier; **FC** = fold change; **FDRP** = first-degree relative participant; **HP** = healthy participant; **IFN-γ** = interferon-γ; **IL** = interleukin; **MS** = multiple sclerosis; **MSP** = multiple sclerosis participant; **TGF-β** = transforming growth factor-β; **Th** = T helper; **VDR** = vitamin D receptor; **VDRE** = vitamin D response element.

There are 2 theories that attempt to elucidate the process of inflammation in patients with multiple sclerosis (MS). First, it is proposed that the disease may rely on the dysregulation of anti-inflammatory cytokines, such as interleukin-10 (IL-10) and interleukin-4 (IL-4), as well as proinflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin-2 (IL-2), interferon-γ (IFN-γ), and interleukin-1β (IL-1β). These pro- and anti-inflammatory cytokines are produced by T-helper 1 (Th-1) and Th-2 cells, respectively.[1] Proinflammatory cytokines augment the permeability of the blood-brain barrier (BBB), allowing for the demyelization and neurodegeneration of the CNS, whereas anti-inflammatory cytokines quell the production of proinflammatory cytokines.[2,3] Second, besides the incomplete notion of Th-1/Th-2 disruption, other studies have proposed that Th-17 cells and IL-17 family members, such as IL-17A and IL-17F, are involved in the disease process. Specially, IL-17A plays a significant role in the stimulation and secretion of proinflammatory cytokines and in chronic CNS inflammation.[4,5]

The development of MS may begin in individuals who are genetically susceptible.[6] Some studies have revealed that first-degree relatives of patients with MS are a 10–25[6] or 20–40[7] times more likely to develop MS than the general population.

Hence, in the present study, we have proposed a schematic model for the fluctuation of pro- and anti-inflammatory cytokines by vitamin D_3, referred to as the See-Saw model (figure e-1, links.lww.com/NXG/A115). In fact, we assessed the response of these interleukins to supplementation with vitamin D_3, as well as the stabilization and balance of this scheme in all 3 groups. Ultimately, our major aim was to find an appropriate way to ameliorate the intensity of MS in afflicted patients and perhaps prevent the disease in 2 other groups via nutrigenomics, especially in first-degree relatives.

Methods

Standard protocol approvals, registrations, and patient consents

This study was approved by the Ethical Committee of Tabriz University of Medical Sciences, Iran (ethical code: IR.TBZMED.REC.1395.780) and Iranian Registry of Clinical Trials (IRCT201703033655N3). Informed consent forms were obtained from all participants, and they could withdraw from the study by their own decision.

Study design and intervention

The study started on February 19, 2017, and ended on June 10, 2017. All randomized participants completed the trial. Twenty-five participants were randomized to each group through simple random sampling. Allocation to each group was through lottery, and random paper was concealed in draw balls (figure 1), including multiple sclerosis participants (MSPs) as the first group (n = 25), first-degree relative participants (FDRPs) of patients with MS, such as son, daughter, sister, or brother as the second group (n = 25), and healthy participants (HPs) as the third group (n = 25). All groups received 50,000 IU of vitamin D_3 orally (Zahravi Pharmaceutical Co, Tabriz, Iran) every Friday and between lunch meals for 8 weeks. Serum 25-(OH) vitamin D_3 and mRNA expression levels of interleukins were measured before and after supplementation in all groups.

Participants and eligibility criteria

The sample size was estimated based on a previous study in Iran,[8] with an odds ratio (OR) of 6, confidence level of 95%, and power of 80%. It was predicted that 25 persons in each group would be sufficient for the detection of changes in serum parameters and gene expression, using G-power. MSPs and FDRPs were selected from the Ardabil MS society. MSPs were diagnosed by a neurologist, according to the McDonald criteria. HPs were chosen from Ardabil University of Medical Sciences. To be included in the study, HPs were at the age of 30 ± 10 years, able to give blood samples, and willing to take part in the study. Malabsorption, taking medicines that interact with vitamin D_3, calcium, and vitamin D_3 supplementation in the last 30 days, gestation, and lactation were considered exclusion criteria. Demographic and disease characteristics are shown in table 1.

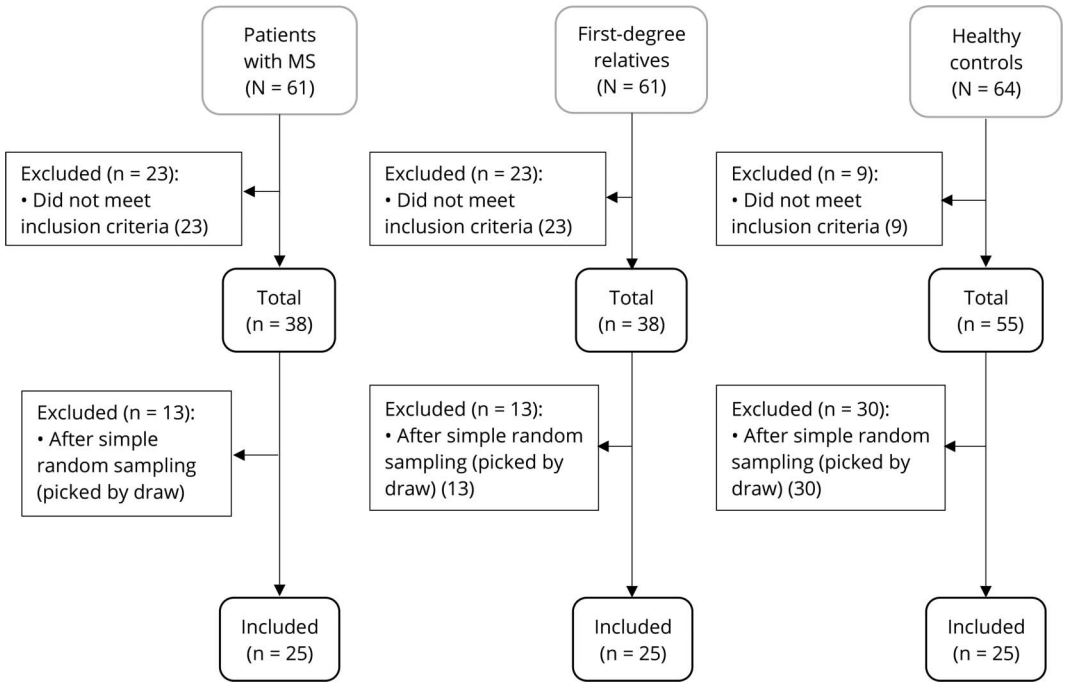
Serum 25-(OH) vitamin D_3 assay

Whole-blood samples (10 mL) were obtained from the participants before and after the trial. To separate the sera, 5 mL of the blood samples was centrifuged at 1,233*g* for 10 minutes at 4°C. Serum levels of 25-(OH) vitamin D3 were measured using electrochemiluminescence assay.

RNA extraction and cDNA synthesis

Five milliliters of blood samples was collected in anticoagulant EDTA tubes. Total RNA was extracted using the MN kit (MACHEREY-NAGEL, Germany), according to the manufacturer's instructions. Concentration, integration, and purity of RNA samples were determined by spectrometry, NanoDrop (Thermo Scientific, Waltham, MA), and gel electrophoresis. Five micrograms of total RNA was used for cDNA synthesis with a random hexamer primer through HyperScript Reverse Transcriptase (GeneAll, South Korea) in 20 μL total reaction mixture.

Figure 1 Enrollment and selection of participants allocated to groups by simple random sampling



Real-time PCR analysis

Real-time PCR was performed on a Roche Light cycler 96 (version: 1.1.0.1320, Germany), using primers specific for IL-6, IL-10, and IL-17A, with β-actin as a housekeeping control. Primer and probe sequences are presented in table 2.[9–12]

Table 1 Demographic and disease characteristics

Variables	MSP (n = 25)	FDRP (n = 25)	HP (n = 25)
Sex[a]			
Female	21 (84%)	17 (68%)	20 (80%)
Male	4 (16%)	8 (32%)	5 (20%)
Age (y)[b]	32.6 ± 6	27.4 ± 6	31.7 ± 4.3
Duration of disease (y)[b]	8.1 ± 5.8	—	—
MS history[a]			
Yes	5 (20%)	25 (100%)	—
No	20 (80%)	—	—
MS family[a]			
Brother	1 (4%)	5 (20%)	—
Sister	3 (12%)	12 (48%)	—
Daughter	1 (4%)	5 (20%)	—
Son	—	3 (12%)	—

Abbreviations: MSP = multiple sclerosis participant; FDRP = first-degree relative participant; HP = healthy participant.
[a] Data are presented as frequency (percent) for categorical variables.
[b] Data are presented as mean ± SD for numeric normal variables.

Real-time PCR reactions were performed in a total volume of 25 μL containing 4 μL of synthesized cDNA solution, 12.5 μL of RealQ Plus 2x Master Mix for probe (Ampliqon, Denmark), 500 nM of each forward and reverse primers, and 250 nM of the TaqMan probe. The amplification program included a prewarming step (10 minutes at 94°C), denaturation step (94°C for 15 seconds), and an annealing/extension step (60°C for 60 seconds).

Outcome measures

The primary outcome of the study was to identify any changes in the serum levels of 25-(OH) vitamin D_3 or in the levels of IL-17A, IL-10, and IL-6 mRNA expression in all 3 groups after 8 weeks.

Adverse events

Participants were continuously monitored for any side effects of vitamin D_3 consumption from baseline of the study. However, no particular events were found.

Statistical analysis

Statistical analysis was performed using SPSS version 23.0 software (SPSS Inc, Chicago, IL). Data are presented using mean ± SD for numeric normal variables and frequency (percent) for categorical variables. The one-way analysis of variance (ANOVA), followed by the post hoc Tukey test, was used for group comparisons. The Paired *t* test was applied for within-group comparisons. Relative mRNA expression normalized to β-actin was calculated by the ΔΔCT method,[13] and the fold change (FC) expression of each gene was calculated by the ratio formula (ratio = $2^{-\Delta\Delta CT}$). In all analysis, $p < 0.05$ were considered statistically significant.

Table 2 Primers and probe sequences used for real-time PCR

Target	Primer
IL-17A[9]	
Forward	AATCTCCACCGCAATGAGGA
Reverse	ACGTTCCCATCAGCGTTGA
Probe	FAM-CGGCACTTTGCCTCCCAGATCACA
IL-10[10]	
Forward	TGAGAACAGCTGCA CCCACTT
Reverse	GCTGAAGGCATCTCGGAGAT
Probe	FAM- CAGGCAACCTGCCTAACATGCTTCGA
IL-6[11]	
Forward	GGTACATCCTCGACGGCATCT
Reverse	GTGCCTCTTTGCTTTCAC
Probe	FAM-TGTTACTCTTGTTACATGTCTCCTTTCTCAGGGCT
Beta-actin[12]	
Forward	TCACCCACACTGTGCCCATCTACGA
Reverse	CAGCGGAACCGCTCATTGCCAATGG
Probe	FAM-ATGCCCTCCCCCATGCCATC

Data availability

The datasets applied and analyzed during the current study are available from the corresponding and first authors on reasonable request from any qualified investigator.

Results

The flow diagram of the study is shown in figure e-2 (links.lww.com/NXG/A116). Of 186 participants, 75 participants (25 per each group) were eligible to be assigned to the study intervention (figure 1).

Association between vitamin D_3 treatment and mRNA expression levels of IL-6, IL-17A, and IL-10

Between-group comparisons

Before supplementation

At baseline, the results of one-way ANOVA showed that there were differences in the mRNA expression levels of IL-6, IL-17A, and IL-10 ($p < 0.001$ for all) among the groups, but levels of serum vitamin D_3 ($p = 0.063$) were almost the same in all groups. The results of the pairwise comparison using the Tukey test also revealed differences between each 2 groups in IL-6 (MSPs and FDRPs: $p < 0.001$, FDRPs and HPs: $p < 0.001$, MSPs and HPs: $p < 0.001$), IL-17A (MSPs and FDRPs: $p = 0.009$, FDRPs and HPs: $p = 0.025$, MSPs and HPs: $p < 0.001$), and IL-10 (MSPs and FDRPs: $p < 0.001$, FDRPs and HPs: $p < 0.001$, MSPs and HPs: $p < 0.001$) (figures 2, A, C, and E and table 3).

After supplementation

After 8 weeks of supplementation with vitamin D_3, there were differences among groups in mRNA expression levels of IL-6 ($p < 0.001$), IL-17A ($p < 0.001$), and IL-10 ($p < 0.001$), as well as in the levels of serum vitamin D_3 ($p = 0.004$). Pairwise comparisons indicated that there were differences between each 2 groups in IL-6 (MSPs and FDRPs: $p < 0.001$, FDRPs and HPs: $p < 0.001$, MSPs and HPs: $p < 0.001$), IL-17A (MSPs and FDRPs: $p = 0.022$, FDRPs and HPs: $p = 0.001$, MSPs and HPs: $p < 0.001$), IL-10 (MSPs and FDRPs: $p = 0.005$, FDRPs and HPs: $p < 0.001$, MSPs and HPs: $p < 0.001$), and serum vitamin D_3 (MSPs and FDRPs: $p = 0.022$, FDRPs and HPs: $p = 0.887$, MSPs and HPs: $p = 0.006$). However, no significant differences were found in the levels of serum vitamin D3 between the FDRP and HP groups (figures 2, B, D, and F and table 3).

Within-group comparisons (before–after)

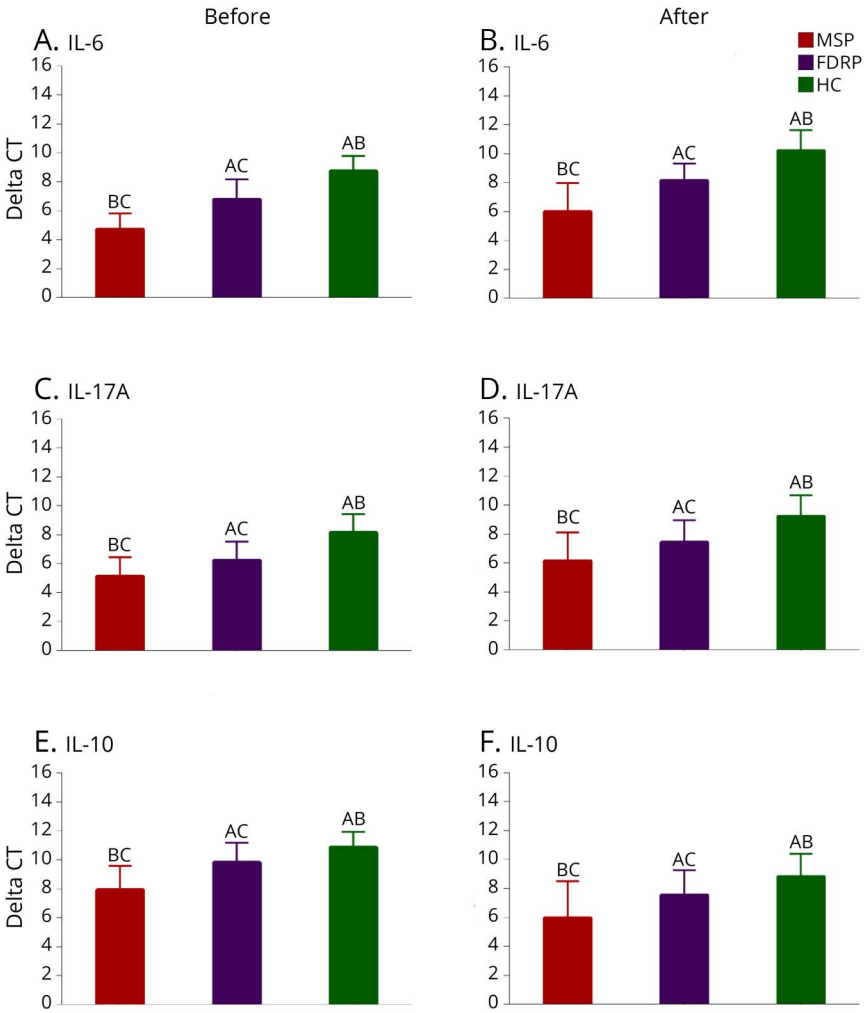
The results of the paired *t* test analyses revealed that the production of proinflammatory cytokines (i.e., IL-17A and IL-6) decreased, whereas secretion of the anti-inflammatory cytokine, IL-10, increased in each group after intervention. The observed FCs in MSPs were −5.9 for IL-17A, ($p = 0.014$), −5.6 for IL-6 ($p = 0.003$), and 6.2 for IL-10 ($p = 0.005$). The FCs in FDRPs were −5.2 for IL-17A ($p = 0.006$), −5.5 for IL-6 ($p = 0.002$), and 4.6 for IL-10 ($p < 0.001$). In HPs, the FCs were −4.2 for IL-17A ($p = 0.012$), −5.1 for IL-6 ($p < 0.001$), and 5.2 for IL-10 ($p < 0.001$) (figures 3, A–C and table 3). In addition, the ratio of pro- to anti-inflammatory cytokines, including the ratio of IL-17A to IL-10 and IL-6 to IL-10, in each group showed that there were differences within the groups (MSPs, $p < 0.001$; FDRPs, $p < 0.001$; HPs, $p < 0.001$, figure 3, D and E).

Discussion

With respect to the role of vitamin D_3 in the immune system and gene expression, we proposed a schematic balance for fluctuations of pro- (IL-17A and IL-6) and anti-inflammatory (IL-10) cytokines by vitamin D_3 that we refer to as the See-Saw model.

In the present trial, 88% of MSPs, 84% of FDRPs, and 80% of HPs showed a positive response after 8 weeks supplementation with 50,000 IU vitamin D_3. There were no differences in serum vitamin D_3 levels among the groups at baseline. This finding is similar to the results of other studies.[14,15] However, our finding does not conform with that of other studies, which indicated that serum levels of vitamin D_3 in MSPs were lower than those in HPs.[16,17] This contradiction may be justified by several reasons, including economic conditions, clothing, inappropriate food habits, and above all, weather conditions and geographical location (the mountainous weather of Northwest of Iran with higher latitude and lower temperature). These may all cause lower exposure to sunlight and consequent insufficiency of serum vitamin D_3. Several studies indicate that vitamin D_3 deficiency is a common phenomenon

Figure 2 Between-group comparisons



Effects of vitamin D_3 supplementation on serum levels of proinflammatory and anti-inflammatory markers (n = 25 per group). (A–F) IL-6, IL-17A, and IL-10 mRNA expression levels of healthy controls (HCs), multiple sclerosis participants (MSPs), and first-degree relative participants (FDRPs). HPs (A), MSPs (B), and FDRPs (C). One-way analysis of variance, followed by the post hoc Tukey test, was used. Data were expressed as mean ± SD, and $p < 0.05$ was regarded as statistically significant.

among patients with MS with neuroinflammatory lesions.[18] In addition, numerous studies have demonstrated the effects of 1, 25-$(OH)_2$ vitamin D_3 on antigen-presenting cell (APC) function and T-cell responses. Vitamin D_3 directly increases the expression level of vitamin D receptor (VDR), especially after bioactivation on T cells.[19] Duan et al.[20] reported that VDR is the active form of vitamin D receptor and is widely expressed in the brain. Evidence suggests that VDRs and vitamin D are key molecules for the development of the nervous system, and they can reduce anxiety and increase the production of neurotrophic factors.

1,25-$(OH)_2$ vitamin D_3 can take part in the activation or repression of gene transcription and expression through direct interaction with VDR/retinoid X receptor (RXR). By this mechanism, 1,25-$(OH)_2$ vitamin D_3 is able to bind directly to specific DNA sequences called vitamin D response elements (VDREs) in target genes.[21]

Our findings indicate that administration of vitamin D_3 decreased the mRNA expression levels of IL-17A and IL-6 and increased the mRNA expression level of IL-10 in all 3 groups. Of 25 participations in each group, we observed downregulation of IL-17A in 76% of MSPs, 80% of FDRPs, and 80% of HPs; we also observed that downregulation of IL-6 in 80% of MSPs, 80% of FDRPs, and 84% of HPs occurred after 8-week treatment with 50,000 IU vitamin D_3. In addition, 80% of MSPs, 88% of FDRPs, and 84% of HPs showed upregulation of IL-10.

IL-17A is produced by Th17 cells, which have a major function in the progression of immune system disorders including MS.[22] Luchtman et al. demonstrated an increase in the secretion of IL-17 in the periphery and in the CNS of patients with MS. IL-17 can lead to increased CCL2, IL-6, and IL-8 secretion. In addition, IL-17 production also results in the formation of reactive oxygen species.[23] Upregulation of IL-6 occurs during neuroinflammation of the CNS and leads to neuronal damage, especially in axons.[24] Furthermore, the presence of transforming growth factor-β (TGF-β) and IL-6 stimulates T-cell differentiation into Th-17 cells, which subsequently increases the level of IL-17A secretion.[8,24] IL-6

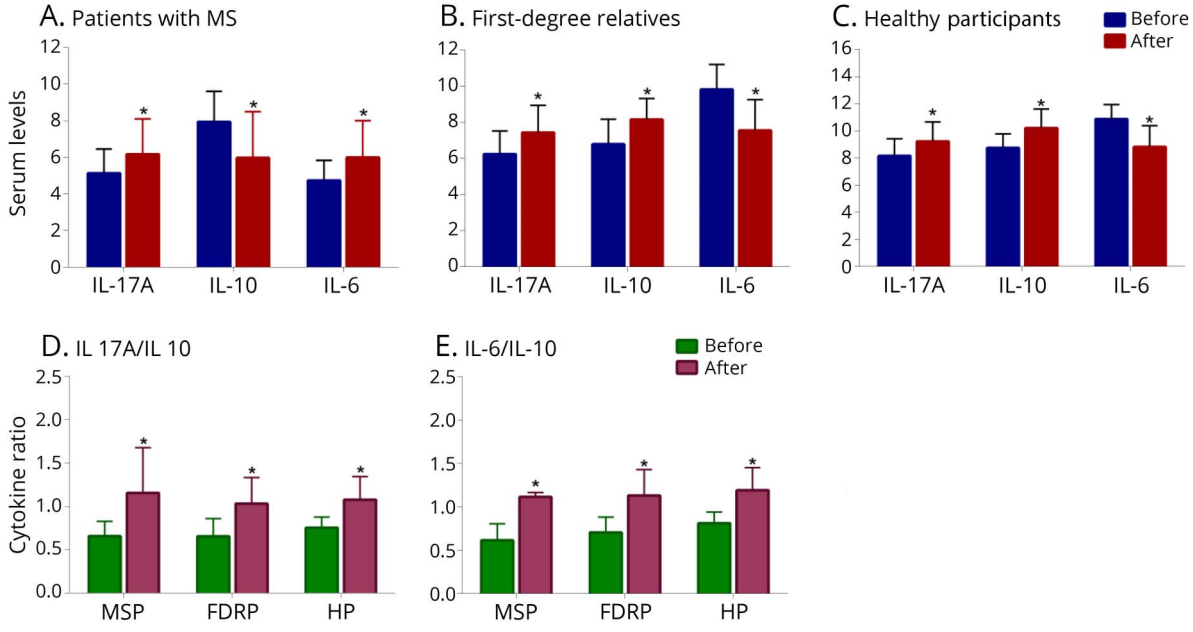
Table 3 Interleukin ΔCT and vitamin D_3 serum levels at baseline and after 8 weeks of supplementation

Variables	MSP (n = 25)	FDRP (n = 25)	HP (n = 25)	*p**	*p*[a]
Before supplementation					
Vitamin D_3 (mean ± SD), *p***	25.94 ± 9.49, BC = 0.067	18.46 ± 12.09, AC = 0.890	19.98 ± 13.07, AB = 0.174	0.063	—
IL-17A (mean ± SD), *p***	5.13 ± 1.32, BC = 0.009	6.24 ± 1.28, AC ≤ 0.001	8.16 ± 1.26, AB ≤ 0.001	<0.001	—
IL-10 (mean ± SD), *p***	7.93 ± 1.66, BC ≤ 0.001	9.82 ± 1.38, AC = 0.025	10.87 ± 1.07, AB ≤ 0.001	<0.001	—
IL-6 (mean ± SD), *p***	4.73 ± 1.09, BC ≤ 0.001	6.79 ± 1.38, AC ≤ 0.001	8.74 ± 1.04, AB ≤ 0.001	<0.001	—
After supplementation					
Vitamin D_3 (mean ± SD), *p*, *p******	62.24 ± 26.07, BC = 0.022, <0.001	46.33 ± 18.72, AC = 0.887, <0.001	43.60 ± 15.86, AB = 0.006, <0.001	0.004	—
IL-17A (mean ± SD), *p*, fold changes,[b] *p******	6.16 ± 1.93, BC = 0.022, −5.9, 0.014	7.43 ± 1.51, AC = 0.001, −5.2, 0.006	9.23 ± 1.44, AB ≤ 0.001, −4.2, 0.012	<0.001	<0.001
IL-10 (mean ± SD), *p*, fold changes, *p******	5.97 ± 2.52, BC = 0.016, 6.2, 0.005	7.55 ± 1.70, AC = 0.067, 4.6, <0.001	8.82 ± 1.56, AB ≤ 0.001, 5.2, <0.001	<0.001	<0.001
IL-6 (mean ± SD), *p*, fold changes, *p******	5.99 ± 2.00, BC ≤ 0.001, −5.6, 0.003	8.15 ± 1.17, AC ≤ 0.001, −5.5, 0.002	10.21 ± 1.40, AB ≤ 0.001, −5.1, <0.001	<0.001	<0.001

Abbreviations: ANOVA = analysis of variance; IL = interleukin; MSP = multiple sclerosis participant; FDRP = first-degree relative participant; HP, healthy participant.
Data are presented as mean ± SD. Between-group comparisons were assessed using one-way ANOVA, followed by the post hoc Tukey test. Within-group comparisons of IL-17A, IL-10, and IL-6 levels were performed using the paired *t* test.
*p**, *p***, and *p**** indicate differences between the groups, pairwise comparisons (Tukey test), and within-group differences, respectively. A, B, and C show HPs, MSPs, and FDRPs, respectively.
[a] ‡*p* indicates the ratio of pro- to anti-inflammatory analysis. $p < 0.05$ indicates statistical significance.
[b] Indicates the expression levels of ILs, compared with baseline values, based on the ratio formula ($2^{-\Delta\Delta CT}$).

plays a key role in MS development in 2 ways: by increasing IL-17 and IL-22 secretion and through the upregulation of CCR6 expression on activated myelin-specific T cells. Therefore, it seems that IL-17A and IL-6 act as agonists through these pathways.[25,26] Da Costa et al.[27] reported that IL-6 and IL-17A levels directly correlated with neurologic

Figure 3 Within-group comparisons (before–after)



Effects of vitamin D_3 on serum levels of proinflammatory and anti-inflammatory markers (n = 25 per group) (A–C) and the ratio of pro-to anti-inflammatory cytokines (D and E). IL-6, IL-17A, and IL-10 mRNA expression levels of healthy participants, patients with MS, and first-degree relatives. Paired *t* test analysis was used. Data were expressed as mean ± SD. *$p < 0.05$ was regarded as statistically significant.

disabilities in patients with MS. Moreover, 1,25-$(OH)_2$ vitamin D_3 decreases the secretion of proinflammatory cytokines in vitro. Naghavi Gargari et al.[8] indicated a positive association between the mRNA expression of IL-6 and IL-17A. Supplementation of 1,25-$(OH)_2$ vitamin D_3 inhibits IL-17A production in T cells of patients with MS.[28] In fact, vitamin D_3 administration can ameliorate MS disease through to downregulation of IL-17A and IL-6. On the other hand, a pivotal role of anti-inflammatory cytokines, especially IL-10, stimulated by vitamin D_3, has been established in the suppression of T-cell activation through macrophages. Therefore, the arrest of IL-10 production may exacerbate inflammation.[29,30] Thereby, vitamin D_3 supplementation can increase IL-10 by T cells and reduce the production of IL-6 and IL-17 in both patient and control groups.[31]

Nevertheless, in several studies, contradictory results have also been reported. For instance, Fujita et al. and Niino et al. have both shown that 1,25-$(OH)_2$ vitamin D_3 can downregulate the production of IL-10.[32,33]. In addition, studies by Naghavi Gargari et al. and Smolders et al. have demonstrated the upregulation of IL-6 and IL-17A by vitamin D.[8,34] Meanwhile, Yao et al.[35] have shown that IL-6 has anti- and pro-inflammatory effects and that it can be secreted from various cell types, including lymphocytes, macrophages, and monocytes. Multiple factors, including the variety of cell types studied, duration and dosage of 1,25$(OH)_2$ vitamin D_3 treatment, type of samples, genetic inheritance of patients, and polymorphisms in VDR genes, have all been proposed to account for these contradictory findings as reported in several studies.[8,14,29,36]

In this study, we also compared the ratio of pro- to anti-inflammatory cytokines (IL-17A:IL-10 and IL-6:IL-10). In all groups, both ratios were improved, but the most significant changes occurred in MSPs. In addition, the changes in the ratio of IL-6:IL-10 were found to be more important than the changes in the ratio of IL-17A:IL-10. Therefore, these results may confirm 2 points: First, vitamin D_3 reduces the gene expression levels of IL-6 and IL-17A. The expression level of IL-6 decreases subsequent to the reduction in IL-17A expression, which is why the ratio of in IL-6:IL10 was more tangible. Second, increased expression of IL-10 had a negative feedback effect on the expression levels of IL-17A and IL-6.

To prove the accuracy of the proposed schematic (See-Saw model), the sample size should be increased in each group. In addition, a blank control group (out of the restricted criteria) should be more defined. In this state, a placebo would be administered to determine the actual effects of vitamin D_3 on the expression levels of genes. Overall, the results suggest that long-term supplementation with a lower daily dosage may be beneficial. Furthermore, participants should be assessed at the midpoint of the study to determine the temporal effect of vitamin D_3 supplementation. A reliable questionnaire should also be devised and used for the effect of vitamin D_3 supplements on the behavioral and mental performance of the participants before and after the intervention. The present study and many other similar ones only examined the mRNA expression levels of these cytokines; however, an analysis of cytokine protein expression would be useful to confirm the effect of vitamin D_3. In addition, epigenetic studies may be useful to address some of the contradictions presented by other studies. Epigenetics refers to the modification of gene expression and changes in chromatin structure without alteration of the DNA sequence.[37] Recent studies suggest that interactions between environmental factors and epigenetic parameters are an underlying cause of MS.

Finally, we determined that upregulation of IL-10 and downregulation of IL-17A and IL-6 occur at sufficient serum levels of vitamin D_3. These features were more noticeable in MSPs. As mentioned earlier, IL-17A and IL-6 augment the production of each other. Sufficient serum levels of vitamin D_3 result in the production of IL-10 and have a negative feedback effect on the expression levels of IL-17A and IL-6. Stabilization and proper balance of the schematic See-Saw model by sufficient levels of vitamin D_3 seem to offer a feasible method for protection from MS by dietary modifications.

Author contributions

S. Rafie-Arefhosseini and R. Hashemi proposed the See-Saw schematic model and wrote the study protocol and design. Davar Altafi, a neurologist, diagnosed patients with multiple sclerosis (MS). Reza Hashemi performed RNA extraction, cDNA synthesis, and real-time PCR under the supervision of Seyed Saeed Hosseini-Asl and also performed analysis and interpretation of the results. Reza Hashemi and Mohammad Morshedi performed statistical analysis and interpretation under the supervision of Mohammad Asghari-Jafarabadi. Mohammad Morshedi drew figures using Graph Prism software. Seyed Rafie-Arefhosseini, Reza Hashemi, and Mohammad Morshedi involved in drafting the manuscript and revising it critically for content. All authors have given their final approval of the version to be published.

Acknowledgment

The authors specially thank patients with MS for their participation in the study and the Nutrition Research Center of Tabriz University of Medical Sciences, Tabriz, Iran, and Ardabil University of Medical Sciences and Imam Khomeini Hospital (Genetic Laboratory) and MS Society, Ardabil, Iran, for their financial support.

Study funding

This study was financially supported by the Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran, and Ardabil University of Medical Sciences, Ardabil, Iran. The results of this article were extracted from the MSc. Thesis of Reza Hashemi (Grant no.: 5/D/960060) was registered at Tabriz University of Medical Sciences, Tabriz, Iran.

Disclosure

R. Hashemi, M. Morshedi, M. Asghari Jafarabadi, and D. Altafi report no disclosures. S. Hosseini-Asl has received research

support from Ardabil University of Medical Sciences. S. Rafie-Arefhosseini is an employee of and has received research support from Tabriz University of Medical Sciences. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/NG.

Received January 9, 2018. Accepted in final form July 9, 2018.

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Neurol Genet 2018;4;
DOI 10.1212/NXG.0000000000000278