Impact of supplementation with "multivitamin-mineral" specially formulated to improve fatigue and inflammatory state in patients with multiple sclerosis: A triple-blind, randomized, placebo-controlled trial

Sama Bitarafan1, Elmira Karimi2,3, Abdorreza Naser Moghadas4, Razieh Sadat Kazemi-Mozdabadi4, Zinat Mohammadpour5, Mohammad Ali Sahraian4

1 Iranian Center of Neurological Research, Neuroscience Institute, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
2 Department of Community Nutrition, School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Tehran, Iran
3 Students’ Scientific Research Center, Tehran University of Medical Sciences, Tehran, Iran
4 Multiple Sclerosis Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran
5 Adelaide Medical School, Faculty of Health and Sciences, University of Adelaide, Adelaide, Australia

Keywords
Multiple Sclerosis; Fatigue; Multivitamin Mineral; Cytokine; Randomized Clinical Trial

Abstract

Background: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) with the most common complaint of fatigue. A high number of patients with MS are interested in taking dietary supplements as a complementary therapy. We propose a specially formulated supplement for patients with MS and aim to evaluate its effects on fatigue.

Methods: This study was a triple-blind, randomized, placebo-controlled trial using a stratified randomization method according to sex. 46 eligible patients participated in the study, 23 in the placebo group and 23 in the intervention group. The intervention group received two capsules of multivitamin-mineral (MVM) daily for 3 months.

How to cite this article: Bitarafan S, Karimi E, Naser Moghadas A, Kazemi-Mozdabadi RS, Mohammadpour Z, Sahraian MA. Impact of supplementation with "multivitamin-mineral" specially formulated to improve fatigue and inflammatory state in patients with multiple sclerosis: A triple-blind, randomized, placebo-controlled trial. Curr J Neurol 2020; 19(4): 180-9.
Measurements of fatigue and cytokines were performed in all patients at the baseline and after the 3-month intervention. 

**Results:** Finally, information of 41 participants was used for data analysis. However, fatigue was decreased after supplementation than before, in the intervention group (P = 0.005). There was no significant difference (P = 0.090) between the change of fatigue score in the MVM group (-3.00 ± 4.42) and the control group (-0.40 ± 5.14). Among cytokines, Interleukin 4 (IL-4) significantly increased in the intervention group compared to the placebo (P = 0.030).

**Conclusion:** Our study showed that the present MVM probably could improve the inflammatory state and fatigue in patients with MS.

**Introduction**

Multiple sclerosis (MS) is an immune-mediated inflammatory disease (IMID) causing demyelination and axonal loss in the central nervous system (CNS). The relapsing-remitting MS (RRMS) is the dominant and benign form of the disease with relapse and remission phases. Another form is secondary progressive MS (SPMS), a slowly worsening phase with more disability, which occurs in patients with RRMS over a long period.3

However, the pathogenesis of MS is still under debate, evidence suggests an underlying role for activated lymphocytes including CD4+ T helper, lymphocytes Th1 and Th17, and cytotoxic CD8+ to stimulate inflammation and autoimmunity in the CNS.4 Studies have shown that activated immune cells initially disturb the blood-brain barrier (BBB) and consequently migrate to the CNS. Imported immune cells release several pro-inflammatory mediators, resulting in structural and functional disruption to the CNS.5-8 Numerous studies have shown that RRMS progress to SPMS due to increased pro-inflammatory cytokines. While pro-inflammatory cytokines released from Th17 (IL-17) and Th1 (IFN-γ, TNF-α, and IL-2) have been implicated as mediators of MS progression, the anti-inflammatory cytokines such as IL-4 and IL-10 produced by regulatory T cells (T-regs) have an antagonist effect on inflammatory cells and can control MS progression.9,12

One of the most common problems due to the aforementioned inflammatory states leading to impaired daily life activities and quality of life (QOL) in patients with MS is the overwhelming feeling of physical or psychological exhaustion called fatigue.13 Despite using medical treatments, most patients report mild to severe fatigue. In addition, many patients with MS experiencing any degree of fatigue have an interest in consuming different types of dietary supplements with various contents of vitamins and minerals as complementary therapy.14

Some studies have shown the effects of supplementation with vitamins or minerals on clinical and laboratory outcomes in patients with MS.15-17 Numerous studies have reported that patients with MS improved after separate supplementations with vitamins (A, B, C, D, and E) and minerals (Calcium, Magnesium, and Selenium). These supplements may reduce the biological synthesis of pro-inflammatory and oxidative compounds.18-25 However, it is well known that excessive intake of Iron, Zinc, and Copper must be controlled to avoid increasing inflammatory and oxidative stress processes due to their cumulative effect in patients with MS. Therefore, many studies have yet to be performed to precisely find out the dosage of vitamins and minerals needed to achieve optimal therapeutic response in patients with MS. Based on the evidence available, it appears that a routine use of multivitamin-mineral (MVM) including Iron, Copper, and Zinc is inappropriate for these patients due to high dose intake of these minerals results in detrimental effects.26-28 Currently, there is no such a suitable supplement appropriate to decrease fatigue in patients with MS. Here, we conducted a clinical trial to investigate whether administration of the specially proposed MVM to MS affects the degree of fatigue and inflammatory state in patients with MS.

**Materials and Methods**

**Study design:** The present study was a triple-blind and randomized clinical trial to compare the state of fatigue and inflammatory factors in patients with MS receiving MVM supplements specialized for fatigue treatment with the placebo group. The study was registered on the Iranian Registry of Clinical Trials (IRCT) with code IRCT2016022026658N1. Additionally, the ethical approval was received from the Ethics Committee, Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.REC.1394.873). 

**Participants:** Randomization was performed using a stratified randomization method according to sex to select eligible patients among those referred to MS Research Center, Sina Hospital, Tehran between December 2018 and June 2019. All the colleagues that were involved in the study were blind, and only one person who coded the drug packs was aware of the intervention who had no further involvement in the study (Figure 1).
The authors checked the inclusion and exclusion criteria, generated the random allocation sequence, and assigned participants to the groups. All participants were informed and signed informed consent forms and they were enrolled with the definite diagnosis of MS (RRMS subtype), according to the 2010 McDonald criteria.29

To control the selection bias, the study was designed to recruit both male and female patients aged 18 to 45 years, which were not in the acute phase of MS at the time of screening. The patients were followed up every month during the study to determine the adhesion to using the supplement. Furthermore, patients were recruited for study if they had all the following inclusion criteria:

- Suffering from RRMS;29
- Receiving interferon within at least 3 months prior to taking part in the study;
- Patients with the same protocol for fatigue treatment [they all received selective serotonin reuptake inhibitors (SSRIs)];
- Lack of taking complementary supplements at least within 3 months prior to taking part in the study;
- Having an Expanded Disability Status Scale (EDSS) score between 0 and 6;
- Patients were excluded from the study if they had one or more of the following criteria:
  - Pregnant participants;
  - Presenting acute forms of liver disease and biliary system and pancreas disease;
  - A history of viral illnesses, asthma, and other autoimmune diseases that have an impact on Th1/Th2 balance, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes (T1D) and inflammatory bowel disease (IBD);
  - Patients with Iron deficient;
  - Participants who had a special diet or changed their diet during the study;
  - Consumption of any nutritional supplements or new drugs during the study;
  - Forgetfulness to use the supplement for more than 12 days (10% of treatment period);
  - Obese patients [body mass index (BMI) ≥ 30 kg/m²], malnourished patients (BMI < 18.5 kg/m²) and substance, cigarette, or alcohol dependence;

*Intervention:* Our intervention group received
a daily intake of two MVM capsules for 3 months. The MVM and placebo capsules were similar in appearance to inhibit the possible information bias.

Each two MVM capsules consisted of 350 μg of vitamin A, 15 μg of vitamin D, 75 μg of vitamin C, 1.1 mg of vitamin B1, 1.5 mg of vitamin B6, 400 μg of vitamin B9, 2.4 μg of vitamin B12, and also 250 mg of calcium, 160 mg of magnesium, 27 μg of selenium, 200 mg of Q10, and 100 mg of L-carnitine.

The dose of vitamins and minerals was recommended according to the Recommended Dietary Allowance (RDA) intake for vitamins D, C, B1, B6, and B12, and preventing toxicity for vitamins A and E, preventing gastrointestinal complications for calcium, magnesium, and selenium. In the case of L-carnitine and Q10, the recommended dose was based on the amounts suggested in published articles. The MVM capsules were designed to have zero amounts of Zinc, iron, and copper and placebo capsules consisted of sunflower oil.

**Assessment of outcomes**

**Primary outcomes:** We measured disability using EDSS. The EDSS test was performed for each subject to obtain a physical measure of neurological impairment before and at the end of the study. The Fatigue Severity Scale (FSS) measured tiredness or fatigue score. The cytokines including Interferon Gama (INF-γ), IL-17 (interleukin), IL-4, and Tumor Necrosis Factor alpha (TNF-α) were performed in all patients at the baseline and after the 3-month intervention. The serum levels of cytokines were measured with enzyme-linked immunosorbent assay (ELISA) kits (Bioassay Technology Laboratory, China).

**Secondary outcomes:** Demographic characteristics including age, sex, smoking, and alcohol use were documented based on self-reported information. Moreover, measurements of anthropometric indices including weight, height, and BMI were evaluated.

To offset any changes in energy and macronutrient consumption (protein, fat, and carbohydrate) that could alter outcomes, standardized 24-hour dietary recalls were recorded through interviews at the baseline and on the same day of the week following the final assessment. Mean daily intakes of energy and nutrients were calculated with computerized Nutri4 software.

The Beck Depression Inventory (BDI) was used to evaluate the degree of depression. The FSS and BDI questionnaires were completed for each participant at the time of enrollment and at the end of the study. Measurements of serum levels of vitamin D, alanine transaminase (ALT), aspartate transaminase (AST), Zinc, Ferritin, and high-sensitivity C-reactive protein (hs-CRP) were performed in all patients at the baseline and after the 3-month intervention, as follows:

**Measurement of hs-CRP in serum** was performed with turbidometric assay by specific ELISA kits and serum concentrations of ALT and AST were measured by enzymatic spectrophotometry using specific ELISA kits (Pars Azmoon, Tehran, Iran and Autoanalyzer BT 1500, Medsystem, USA). Serum Zinc was determined by enzymatic spectrophotometry with specific ELISA kits (Zist Shimi, Tehran, Iran and Autoanalyzer BT 1500, Medsystem, USA). Serum 25 (OH) vitamin D and ferritin levels were estimated using electrochemiluminescence (ECL) and an ELISA kit provided by Roche Diagnostics GmbH (6506780160, Mannheim, Germany) by an automated device (Cobas e411; Roche Diagnostics GmbH, Mannheim, Germany).

The data were screened for normality through the one-sample Kolmogorov-Smirnov (KS) test. Furthermore, parametric and non-parametric tests were applied to analyze the data with normal and non-normal distributions, respectively. A paired sample t-test was employed to compare the intragroup discrepancies in fatigue state and biochemical markers, before and after the intervention. An independent sample t-test was also used for assessing differences of the mentioned outcomes between groups before and after the intervention. All statistical analyses were performed using Statistical Package for Social Science (version 18.0, SPSS Inc., Chicago, IL, USA) and P-values < 0.050 were considered statistically significant.

**Results**

**Recruitment and baseline characteristics:** 46 eligible patients with MS were enrolled in the study, 23 patients to the MVM group, and 23 patients to the placebo group. 2 patients were excluded from the intervention group; 1 did not participate in the follow-up tests due to emigration and the other patient had low compliance (remaining tablets > 10% of the total). Moreover, 3 patients in the placebo group withdrew from the study due to severe hair loss. Finally, information of 41 remaining participants was used for data analysis. The primary characteristics of participants are presented in Table 1. There was no significant difference between the two groups in age, sex, duration of illness, energy intake, and BMI (Table 1).
Table 1. Self-explanatory characteristics of patients

| Characteristic                          | Groups                  | P       |
|-----------------------------------------|-------------------------|---------|
|                                         | Supplementation         | Placebo |
| Sex (female/male) [n (%)]               | 21 (17.4)               | 20 (17.3)| 0.530*  |
| Age (mean ± SD)                         | 35.14 ± 5.39            | 35.35 ± 5.73 | 0.910*  |
| Disease duration (mean ± SD)            | 7.10 ± 3.18             | 6.70 ± 3.88 | 0.720*  |
| BMI (mean ± SD)                         | 24.49 ± 1.90            | 23.76 ± 1.65 | 0.200*  |
| Energy intake** (mean ± SD)             | 1930.29 ± 111.20        | 1958.85 ± 112.70 | 0.410*  |

BMI: Body mass index; SD: Standard deviation
*Independent sample t-test, **Kilocalorie/day, #Chi-square

Blood biochemical outcomes: The serum levels of biochemical factors including ALT, AST, Zinc, vitamin D, Ferritin, and CRP in the two groups before and after the study are summarized in table 2. There were no significant differences in these biochemical markers between the two groups, but there was a significant reduction in ALT (P = 0.040) and hs-CRP (P = 0.006) in the intervention group after the study. In addition, a significant decrease was observed in ferritin concentration in both groups after the study (Table 2).

BMI and energy intake: Comparisons of BMI and intake calories between intervention and control groups before and after the study are summarized in Table 3. The change in BMI had a significant reduction in the intervention group compared to the placebo group. However, the change in calorie intake was not significantly different between the two groups (Table 3).

Clinical outcomes: However, there was no significant difference between the groups (P = 0.090); the intervention group reported less fatigue experience after supplementation compared to before the supplementation (P = 0.005). Furthermore, neither depression (P = 0.180) nor disability rates (P = 0.110) were not different between the groups (Table 3).

Serum levels of cytokines: The present findings showed an increase in IL4 in the intervention group compared to the placebo group (P = 0.030). Serum levels of the other cytokines did not differ between the groups. However, after the 3-month intervention, the INF-γ level in the intervention group decreased significantly after the intervention compared to before (P = 0.040) (Table 4).

Table 2. Comparison of biochemical parameters between the two groups of study and between before and after supplementation in each group

| Biochemical parameters | Time | Groups† | P       |
|------------------------|------|---------|---------|
|                        |      | Supplementation | Placebo |         |
| Aspartate transaminase | Before | 22.95 ± 8.60 | 21.10 ± 8.73 | 0.370  |
|                        | After  | 21.29 ± 6.89 | 20.90 ± 8.03 | 0.870  |
|                        | Change | -1.67 ± 4.78 | -0.20 ± 4.81 | 0.520  |
|                        | P***   | 0.480      | 0.610    |         |
| Alanine transaminase   | Before | 21.33 ± 13.38 | 21.35 ± 17.70 | 0.570  |
|                        | After  | 17.05 ± 7.66 | 21.95 ± 11.95 | 0.130  |
|                        | Change | -4.29 ± 9.128 | 0.60 ± 10.97 | 0.130  |
|                        | P**    | 0.040      | 0.810    |         |
| Zinc                   | Before | 146.80 ± 34.65 | 144.48 ± 18.84 | 0.790  |
|                        | After  | 134.44 ± 14.51 | 142.64 ± 15.55 | 0.090  |
|                        | Change | -12.36 ± 28.41 | -1.84 ± 5.74 | 0.490  |
|                        | P***   | 0.110      | 0.190    |         |
| Vitamin D              | Before | 61.78 ± 26.96 | 53.27 ± 25.91 | 0.310  |
|                        | After  | 61.78 ± 25.79 | 54.55 ± 25.85 | 0.380  |
|                        | Change | -0.01 ± 8.08 | 1.29 ± 5.14 | 0.550  |
|                        | P**    | 0.990      | 0.270    |         |
| Ferritin               | Before | 125.28 ± 86.57 | 105.75 ± 80.87 | 0.460  |
|                        | After  | 94.89 ± 65.37 | 94.78 ± 72.76 | 0.980  |
|                        | Change | -30.40 ± 37.46 | -10.96 ± 15.63 | 0.137  |
|                        | P***   | < 0.001    | 0.002    |         |
| CRP                    | Before | 2.82 ± 2.27 | 2.80 ± 1.97 | 0.880  |
|                        | After  | 2.31 ± 1.84 | 2.44 ± 1.86 | 0.690  |
|                        | Change | -0.51 ± 0.77 | -0.37 ± 0.88 | 0.570  |
|                        | P**    | 0.006      | 0.080    |         |

SD: Standard deviation CRP: C-reactive protein
†Mean ± SD, †Independent sample t-test, †Paired sample t-test, ††Wilcoxon signed-rank test, †††Mann-Whitney U test
Table 3. Comparison of clinical outcomes between the two groups of study and between before and after supplementation in each group

| Clinical outcomes | Time | Groups | P* |
|-------------------|------|--------|----|
|                   |      | Supplementation | Placebo |
| BMI               | Before | 24.49 ± 1.90 | 23.76 ± 1.65 | 0.200 |
|                   | After  | 24.08 ± 1.84 | 23.74 ± 1.64 | 0.540 |
|                   | Change | -0.42 ± 0.48 | -0.02 ± 0.24 | 0.002 |
|                   | P***   | 0.001 | 0.620 |
| Calorie          | Before | 1930.29 ± 111.20 | 1958.85 ± 112.70 | 0.420 |
|                   | After  | 1937.81 ± 110.95 | 1939.15 ± 127.11 | 0.970 |
|                   | Change | 7.52 ± 95.93 | -19.70 ± 63.21 | 0.290 |
|                   | P**    | 0.720 | 0.180 |
| Fatigue          | Before | 38.52 ± 8.01 | 36.20 ± 6.46 | 0.310 |
|                   | After  | 35.52 ± 8.23 | 35.80 ± 6.72 | 0.910 |
|                   | Change | -3.00 ± 4.42 | -0.40 ± 5.14 | 0.090 |
|                   | P**    | 0.640 | 0.730 |
| Depression       | Before | 18.14 ± 3.32 | 17.60 ± 3.49 | 0.610 |
|                   | After  | 17.48 ± 7.09 | 19.25 ± 5.25 | 0.370 |
|                   | Change | -0.67 ± 6.42 | 1.65 ± 4.08 | 0.180 |
|                   | P**    | 0.640 | 0.090 |
| Disability       | Before | 1.33 ± 1.32 | 1.20 ± 1.32 | 0.610 |
|                   | After  | 1.24 ± 1.18 | 1.30 ± 1.38 | 0.930 |
|                   | Change | -0.10 ± 0.30 | 0.10 ± 0.45 | 0.110 |
|                   | P***   | 0.160 | 0.320 |

BMI: Body mass index; SD: Standard deviation
¥Mean ± SD, *Independent sample t-test, **Paired sample t-test, ***Wilcoxon signed-rank test, 4Mann-Whitney U test; €Kilocalorie/day

Discussion

Despite the high prevalence of fatigue and its detrimental impact on the QOL of patients with MS, few studies have been conducted to address this issue. The effects of complementary treatment for fatigue improvement in patients with MS have remained uncertain. One strong hypothesis expresses that the severity of fatigue is dependent on the inflammation situation in patients with MS. In this way, supplementation with vitamins, minerals, and MVM could be a safe complementary therapeutic option that reduces the severity of fatigue by improving the inflammatory status.

Table 4. Comparison of Inflammatory indexes between the two groups of study and between before and after supplementation in each group

| Inflammatory indexes | Time | Groups | P* |
|----------------------|------|--------|----|
|                      |      | Supplementation | Placebo |
| IL-4                | Before | 58.77 ± 71.05 | 54.31 ± 44.87 | 0.120 |
|                     | After  | 84.23 ± 111.99 | 43.94 ± 36.87 | 0.970 |
|                     | Change | 25.46 ± 52.34 | -10.37 ± 32.82 | 0.030 |
|                     | P***   | 0.090 | 0.110 |
| IL-17               | Before | 33.10 ± 38.50 | 33.57 ± 37.73 | 0.270 |
|                     | After  | 32.88 ± 37.69 | 35.80 ± 36.42 | 0.220 |
|                     | Change | -0.22 ± 3.06 | 2.01 ± 6.30 | 0.160 |
|                     | P**    | 0.740 | 0.170 |
| TNF                 | Before | 336.28 ± 164.24 | 315.03 ± 150.12 | 0.710 |
|                     | After  | 318.21 ± 164.24 | 312.41 ± 155.38 | 0.970 |
|                     | Change | -18.07 ± 40.93 | -2.63 ± 23.42 | 0.150 |
|                     | P**    | 0.060 | 0.620 |
| INF-γ               | Before | 35.24 ± 54.48 | 24.28 ± 32.45 | 0.520 |
|                     | After  | 25.16 ± 42.18 | 24.95 ± 32.33 | 0.880 |
|                     | Change | -10.08 ± 16.18 | 0.67 ± 3.46 | 0.070 |
|                     | P***   | 0.040 | 0.850 |

SD: Standard deviation; IL: Interleukin; TNF: Tumor Necrosis Factor; INF-γ: Interferon Gama
¥Mean ± SD, *Independent sample t-test, **Paired sample t-test, ***Wilcoxon signed-rank test, 4Mann-Whitney U test
On the other hand, evidence has shown the adverse cumulative effects of the continuous intake of some minerals such as Iron, Copper, and Zinc through MVM supplements in patients.\textsuperscript{26-28,42-44} Therefore, we proposed a specifically formulated MVM without Iron, Copper, and Zinc and aimed to investigate its effect on fatigue and cytokines in patients with MS. Finally, we showed the reductive trend for fatigue and inflammatory markers including CRP and INF-\textgamma as well as a growing trend for IL-4 that is an anti-inflammatory cytokine.

However, our findings are supported by similar studies that have tested common vitamin or mineral supplements as a complementary treatment for MS. For example, improvement in fatigue scale in patients with chronic fatigue syndrome (CFS) as well as level of superoxide dismutase activity was reported after 2 months of MVM supplements.\textsuperscript{45,46} Another study reported that short-term exposure to MVM intervention in older adults resulted in reduced circulating level of CRP and oxidative stress.\textsuperscript{47} In addition, Johnson et al. found that one supplement containing magnesium, zinc, selenium, and vitamins B6, D, A, and E could reduce MS incidence.\textsuperscript{22}

Moreover, our interpretation was consistent with the finding of a meta-analysis in which the significant benefits of MVM supplementation were reported on fatigue.\textsuperscript{48} However, subjects inversely responded to MVM treatment and exhibited a higher inflammatory status. For instance, MVM supplementation led to an unexpected increased oxidative stress in healthy adults that is a contributing factor to inflammation.\textsuperscript{49,50} Studies reported that this discrepancy is likely through Fenton reaction by Iron molecules existing in the supplement.\textsuperscript{51}

Some studies have provided evidence in support of the findings of our study. In this regard, recent studies have shown that retinoid, derived from vitamin A, has been shown to slow down MS progression for even the progressive phase of the disease.\textsuperscript{52} Retinoid directly promotes the pro-inflammatory/anti-inflammatory balance and is associated with transcription of major anti-inflammatory mediators, followed by decreased Th1 and Th17 proliferation.\textsuperscript{52,53} Furthermore, vitamin A has been shown to have a synergistic effect with INF-\beta.\textsuperscript{54}

In our previous study on vitamin A, we provided the long-term beneficial consequences on fatigue severity in patients with MS.\textsuperscript{16} Another fat-soluble vitamin, calcitriol, present in our supplement has the same performance on the immune system as vitamin A.\textsuperscript{55} There has been report of a reduced fatigue state after treatment with a single dose of vitamin D in healthy people.\textsuperscript{55} It is thought that imbalances in the dopamine level in the CNS may motivate fatigue centers in the brain and vitamin D is able to modify this imbalance.\textsuperscript{54} Further mechanisms for the involvement of vitamin D in reducing fatigue rate come from its ability in stimulating the production of the serotonin in the brain,\textsuperscript{56} which has been shown to be inversely related to tiredness.\textsuperscript{57} Moreover, the oral administration of calcium with or without vitamin D acts through various mechanisms, such as reducing inflammatory marker levels (TNF-\alpha, IL-6), through which regulates the immune system.\textsuperscript{53,59} As such, the role that calcium may have on fatigue by reducing these inflammatory markers could present insight into the benefit of its supplementation in MS.

The rationale for using antioxidants in our MVM is based on the knowledge that oxidative stress is one of the most critical components of the MS disease.\textsuperscript{60} Unrestricted reactive oxygen species (ROS) production under chronic inflammatory conditions in MS is responsible for depleting the body’s antioxidant reserves, including vitamins C and E and selenium.\textsuperscript{61}

In this regard, studies showed the protective role of vitamins E and C and selenium in maintaining blood concentrations of glutathione peroxidase and decreasing prostaglandin E2 secretion from macrophages in patients with MS.\textsuperscript{62-64} B vitamins, including B1, B2, B3, B5, B6, and B12 have been shown to play inter-related functions in reducing fatigue severity after physical activity in people living in hot climates.\textsuperscript{65} Some observational studies have also reported that fatigue in patients with MS may be associated with mild intracellular vitamin B1 deficiency and subsequent impairment of thiamine-dependent cell reactions.\textsuperscript{66} Another example of fatigue reducing properties of B vitamins is the role of vitamin B12 in synthesizing and maintaining myelin in patients with MS.\textsuperscript{67} Furthermore, a significant relationship between folic acid deficiency and increased fatigue has been observed in these patients.\textsuperscript{68}

Vitamin B6 and magnesium have a related role in regulating nitric oxide (NO) concentration within vascular endothelial cells and act as coenzymes in regulating intracellular NO production and the secretion of NO from cells. Therefore, deficiency of vitamin B6 or magnesium
leads to NO entrapment inside cells and its subsequent reaction with superoxide which produces nitrogen peroxide, leading to its accumulation and many adverse cellular consequences including myelin destruction.22

Magnesium homeostasis is physiologically linked to other minerals such as zinc, calcium, and aluminum that maintaining their concentration within optimal ranges is crucial for the desired function of both the immune system and CNS.69

Adjunctive supplemented carnitine as well as ubiquinone (Q10) with vitamins and minerals may have benefits in reducing fatigue in patients with MS.70,71

Theoretically, the effect of carnitine is because of its role in energy production in mammalian cells and high excretion of this molecule has been observed in this disease because of stable inflammatory state.

In terms of Q10, the results of a study showed the anti-oxidative feature of this component in subjects with MS followed by reduced inflammation in these patients.72,73

A significant difference was seen between groups in BMI. However, malnutrition often occurs in patients with MS, so reduced BMI may not be desirable in patients with MS with normal weight.74

The probable reason for this finding could be the effect of carnitine presence in the supplement, because of its decreasing effect on weight and BMI that has been proven in various studies.75

Furthermore, the remarkable reduction of ferritin levels was observed in both groups in our study. This could be attributed to the lack of iron in the supplement used in this study in intervention group. Additionally, it was because of our inclusion criteria to cut the supplementation during 3 months before study in the placebo group. Liver enzymes were not affected in the present study. This outcome could be the desired result due to the oxidative characteristics of Iron.

The main strength of our study was being a triple-blind and randomized clinical trial study with a novel MVM formulation. Besides, the confounding effects of depression and disability on fatigue were measured and controlled. There were some limitations in the study; because of the restrictive inclusion criteria, we could not achieve enough sample size in the time specified for the study. One of the limitations was unwanted hair loss in the placebo group that was speculated to be a result of their previous supplementation discontinuation before the study initiation. On the other hand, more trials with larger sample sizes and longer supplementation periods are recommended to provide more certain results.

Conclusion

The present study indicated that the specially formulated MVM for patients with MS could probably improve fatigue and inflammatory state.

Conflict of Interests

The authors declare no conflict of interest in this study.

Acknowledgments

The authors would like to appreciate NanoHayat Darou Company for financial supports and MVM production. The authors are grateful to Tehran University of Medical Sciences and Health Services (Project registration number: 94-02-188-27795) for supervision and confirmation of the study.

References

1. Klots L, Havla J, Schwab N, Hohlfeld R, Barnett M, Reddel S, et al. Risks and risk management in multiple sclerosis immunotherapeutic treatment. Ther Adv Neurol Disord. 2019; 12: 1756286419836571.
2. Tillery EE, Clements JN, Howard Z. What’s new in multiple sclerosis? Ment Health Clin 2017; 7(5): 213-20.
3. Weinschenker BG, Bax B, Rice GP, Noseworthy J, Carriere W, Baskerville J, et al. The natural history of multiple sclerosis: A geographically based study. I. Clinical course and disability. Brain 1989; 112 (Pt 1): 133-46.
4. McFarland HP, Martin R. Multiple sclerosis: A complicated picture of autoimmunity. Nat Immunol 2007; 8(9): 913-9.
5. Zajicek JP, Wing M, Scolding NJ, Compston DA. Interactions between oligodendrocytes and microglia. A major role for complement and tumour necrosis factor in oligodendrocyte adherence and killing. Brain 1992; 115 (Pt 6): 1611-31.
6. Vartanian T, Li Y, Zhao M, Stefansson K. Interferon-gamma-induced oligodendrocyte cell death: Implications for the pathogenesis of multiple sclerosis. Mol Med 1995; 1(7): 732-43.
7. Benveniste EN, Merrill JE. Stimulation of oligodendroglial proliferation and maturation by interleukin-2. Nature 1986; 321(6070): 610-3.
8. Compston A, Coles A. Multiple sclerosis. Lancet 2008; 372(9648): 1502-8.
9. Amedei A, Prisco D, D’Eliaos MM. Multiple sclerosis: The role of cytokines in pathogenesis and in therapies. Int J Mol Sci 2012; 13(10): 13438-60.
10. Sacramento PM, Monteiro C, Dias ASO, Kasahara TM, Ferreira TB, Hygino J, et al. Serotonin decreases the production of Th1/Th17 cytokines and elevates the frequency of regulatory CD4(+) T-cell subsets in multiple sclerosis patients. Eur J Immunol 2018; 48(8): 1376-88.
11. Rodgers JM, Miller SD. Cytokine control of inflammation and repair in the pathology of multiple sclerosis. Yale J Biol Med 2012; 85(4): 447-68.
12. Kleinvieitfeld M, Hafer DA. The plasticity of human Th1 and Th17 cells and its role in autoimmunity. Semin Immunol 2015; 25(4): 305-12.
13. Gumus H. Fatigue Can Be Objectively Measured In Multiple Sclerosis: Multipl
Specified multivitamin and fatigue in MS

Sclerozda Yorgunluk Objetifik Oralar Okulerlebilir. Noro Psikijatr Arsl 2018; 55(Suppl 1): S76-S79.

14. Masullo L, Papas MA, Cotugna N, Baker S, Mahoney L, Traubli J. Complementary and alternative medicine use and nutrient intake among individuals with multiple sclerosis in the United States. J Community Health 2015; 40(1): 153-60.

15. Xue H, Ren H, Zhang L, Sun X, Wang W, Zhang S, et al. Alpha-tocopheryl ameliorates experimental autoimmune encephalomyelitis through the regulation of Th1 cells. Iran J Basic Med Sci 2016; 19(5): 561-6.

16. Bitarafan S, Saboor-Yarahgi A, Sahraian MA, Soltani D, Nafissi S, Togha M, et al. Effect of Vitamin A Supplementation on fatigue and depression in Multiple Sclerosis patients: A double-blind placebo-controlled clinical trial. Iran J Allergy Asthma Immunol 2016; 15(1): 13-9.

17. Naghashpour M, Majdinasab N, Shakerinjejad G, Kouchak M, Haghhighazdeh MH, Jarvandi F, et al. Riboflavin supplementation to patients with multiple sclerosis does not improve disability status nor is riboflavin supplementation correlated to homocysteine. Int J Vitam Nutr Res 2013; 83(5): 281-90.

18. Springman SR, Bleijenberg G, Bazelmans E, Elving LD, de Boo TM, Severens JL, et al. Cognitive behaviour therapy for chronic fatigue syndrome: A multicentre randomised controlled trial. Lancet 2001; 357(9259): 841-7.

19. Jafari Rad S, Sima M, Harirchian MH, Sahraian MA, Eshraghi MR, Shokri F, et al. The effect of vitamin A supplementation on stimulated T-cell proliferation with multiple sclerosis in patients with multiple sclerosis. J Neurol Sci Rural Pract 2012; 3(3): 294-8.

20. Wikstrom J, Westermarck T, Palo J. Selenium, vitamin E and copper in multiple sclerosis. Acta Neurol Scand 2003; 107(6): 287-90.

21. Bottiglieri T, Folate, B12, and neuropsychiatric disorders. Nutr Rev 1996; 54(12): 382-90.

22. Johnson S. The possible role of gradual accumulation of copper, cadmium, lead and iron and gradual depletion of zinc, magnesium, selenium, vitamins B2, B6, D, and E and essential fatty acids in multiple sclerosis. Med Hypotheses 2000; 55(3): 239-41.

23. Moore MM. Treatment of multiple sclerosis with nicotinic acid and vitamin B1 preliminary report. Arch Intern Med (Chic) 1940; 65(1): 1-20.

24. Burton JM, Kimball S, Vieith R, Bar-Ora A, DuffY P, Zimime D. Use of alpha tocopherol in multiple sclerosis. II. Correlation with disease activity and elevated plasma membrane-bound zinc in erythrocytes from patients with multiple sclerosis. Ann Neurol 1986; 20(6): 712-5.

25. Melo TM, Larsen C, White LR, Aasly J, Sjobakk TE, Flaten TP, et al. Manganese, copper, and zinc in cerebrospinal fluid from patients with multiple sclerosis. Biol Trace Elem Res 2003; 93(1-3): 1-8.

26. Bloem B, Iron and multiple sclerosis [PhD Thesis]; Stellenbosch, South Africa: University of Stellenbosch; 2007.

27. Sheyhkhansri S, Koziecki K, Bill J, Sitti M, Gemmati D, Zamboni P, et al. Redox metals homoeostasis in multiple sclerosis and amyotrophic lateral sclerosis: A review. Cell Death Dis 2018; 9(3): 348.

28. Sadaka Y, Verhey LH, Shroff MM, Branson HM, Arnold DL, Narayanans S, et al. 2010 McDonald criteria for diagnosing pediatric multiple sclerosis. Ann Neurol 2012; 72(2): 211-23.

29. Embry AF, Snowden LR, Vieth R. Vitamin D and seasonal fluctuations of gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis. Ann Neurol 2000; 48(2): 271-2.

30. Faridar A, Eskandari G, Sahraian MA, Minagar A, Azimi A, Vitamin D and multiple sclerosis: A critical review and recommendations on treatment. Acta Neurol Belg 2012; 112(4): 327-33.

31. Sanooar M, Eghtesadi S, Azimi N, Khalili M, Fazayeri S, Reza GM. Coenzyme Q10 supplementation reduces oxidative stress and increases antioxidant enzyme activity in patients with relapsing-remitting multiple sclerosis. Int J Neurosci 2013; 123(11): 776-82.

32. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: An expanded disability status scale (EDSS). Neurology 1983; 33(11): 1444-52.

33. Krupp LB, LaRocca NG, Muir-Nash J, Scadden, P. The frequency and severity of fatigue in patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989; 46(10): 1121-3.

34. Beck AT, Steer RA, Brown GK. BDI-II, Beck Depression Inventory: Manual. New York, NY: Psychological Corporation; 1996, p. 2.

35. Stroud NM, Minahan CL. The impact of regular physical activity on fatigue, depression and quality of life in persons with multiple sclerosis. Health Qual Life Outcomes 2009; 7: 68.

36. Braley TJ, Chervin RD. Fatigue in multiple sclerosis: Mechanisms, evaluation, and treatment. Sleep 2010; 33(8): 1061-7.

37. Chalab MA, Ayache SS. Is there a link between inflammation and fatigue in multiple sclerosis? J Inflamm Res 2011; 8: 253-64.

38. Hoesen C, Narawath R, Reich C, Bauer N, Schulz KH, Gold SM. Fatigue in multiple sclerosis: An example of cytokine mediated sickness behaviour? J Neurol Neurosurg Psychiatry 2006; 77(1): 34-9.

39. Flachenecker P, Bilker L, Weber F, Gottschalk M, Tzoker K, Rieckmann K. Cytokine mRNA expression in patients with multiple sclerosis and fatigue. Mult Scler 2004; 10(2): 165-9.

40. Schwind SR, Covington M, Segal BM, Goodman AD. Fatigue in multiple sclerosis: current understanding and future directions. J Rehabil Res Dev 2002; 39(2): 211-24.

41. Dickhut L, Bucelkowski A, Muci M, Pitotti E, De Robertis F, Trianni G, et al. Copper and ceruloplasmin dyshomeostasis in serum and cerebrospinal fluid of multiple sclerosis subjects. Biochim Biophys Acta Mol Basis Dis 2018; 1846(5 Pt A): 1828-38.

42. Nachmi AD, Hassan AF, Hammady MM. Estimation the level of metals (Lead, Cadmium, Copper and Zinc) in multiple sclerosis patients in Banar’Iraq. Indian J Forensic Med Toxicol 2020; 14(3): 1029-35.

43. Bredolt M, Frederiksen JL. Zinc in multiple sclerosis: A systematic review and meta-analysis. ASN Neuro 2016; 8(3): 1759091416651151.

44. Marie D, Birkic S, Tomie S, Novakov MA, Cebovic T, Turkulov V. Multivitamin mineral supplementation in patients with chronic fatigue syndrome. Med Sci Monit 2014; 20: 47-53.

45. Bitarafan S, Yin LY, Zhang Y, Fan HJ, Chang JI, Dawsey SM, et al. Multivitamin and mineral supplementation is associated with the reduction of fracture risk and hospitalization rate in Chinese adult males: A randomized controlled study. J Bone Miner Metab 2015; 33(3): 294-302.

46. Harris E, Macpherson H, Pipingas A. Improved blood biomarkers but no cognitive effects from 16 weeks of multivitamin supplementation in healthy older adults. Nutrients 2015; 7(9): 3796-812.

47. Long SJ, Benton D. Effects of vitamin and mineral supplementation on stress, mild psychiatric symptoms, and mood in nonclinical samples: A meta-analysis. Psychosom Med 2013; 75(2): 144-53.

48. Jansen E, Beehkov P, Tomasinius A, Lukkisie D, Bacevicene M. Biomarkers of oxidative stress and redox status in a short-term low-dosed multivitamin and mineral supplementation study in two human age groups. Biogerontology 2015; 16(5): 645-53.

49. Hussain T, Tan B, Yin Y, Blachier F, Tossou MC, Rahu N. Oxidative Stress and inflammation: What polyphenols can do for us? Oxid Med Cell Longev 2016; 2016: 7432797.

50. Meneghini R. Iron homoeostasis, oxidative stress, and DNA damage. Free Radiol Biol Med 1997; 23(5): 783-92.

51. Nowak A, Boesch L, Andres E, Battegay E, Hornermann T, Schmid C, et al. Effect of vitamin D3 on self-perceived fatigue: A double-blind randomized placebo-controlled trial. Medicine (Baltimore) 2016; 95(52): e5353.

52. Azzi A. The role of alpha-tocopherol in preventing disease. Eur J Nutr 2004; 43(Suppl 1): I18-I25.

53. Dobranyova E, Genova HM, DeLaura J, Wylie GR. The dopamine imbalance hypothesis of fatigue in multiple sclerosis and other neurological disorders. Front Neurol 2015; 6: 52.
55. Hoe E, Nathanielsz J, Toh ZQ, Spry L, Marimla R, Balloch A, et al. Anti-inflammatory effects of vitamin D on human immune cells in the context of bacterial infection. Nutrients 2016; 8(12): 806.

56. Kaneko I, Sabir MS, Dussik CM, Whitfield GK, Karrys A, Hsieh JC, et al. 1,25-Dihydroxyvitamin D regulates expression of the tryptophan hydroxylase 2 and leptin genes: Implication for behavioral influences of vitamin D. FASEB J 2015; 29(9): 4023-35.

57. Yamamoto S, Ouchi Y, Onoe H, Yoshikawa E, Tsukada H, Takahashi H, et al. Reduction of serotonin transporters of patients with chronic fatigue syndrome. Neureport 2004; 15(17): 2571-4.

58. Zemel MB, Sun X. Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans. J Nutr 2008; 138(6): 1047-52.

59. Zhu Y, Mahon BD, Froicu M, Cantorna MT. Calcium and 1 alpha,25-dihydroxyvitamin D3 target the TNF-alpha pathway to suppress experimental inflammatory bowel disease. Eur J Immunol 2005; 35(1): 217-24.

60. Sybura C, Passi S. Oxidative stress in patients with multiple sclerosis. Ukr Biokhim Zh (1999) 1999; 71(3): 112-5.

61. Ford ES, Liu S, Mammo DM, Giles WH, Smith SJ. C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. Eur J Clin Nutr 2003; 57(9): 1157-63.

62. Mai J, Sorensen PS, Hansen JC. High dose antioxidant supplementation to Patients with MS. Effects on glutathione peroxidase, clinical safety, and absorption of selenium. Biol Trace Elem Res 1990; 24(2): 109-17.

63. Cohen ME, Meyer DM. Effect of dietary vitamin E supplementation and rotational stress on alveolar bone loss in rice rats. Arch Oral Biol 1993; 38(7): 601-6.

64. Han SN, Wu D, Ha WK, Beharka A, Smith DE, Bender RS, et al. Vitamin E supplementation increases T helper 1 cytokine production in old mice infected with influenza virus. Immunology 2000; 100(4): 487-93.

65. Early RG, Carlson BR. Water-soluble vitamin therapy in the delay of fatigue from physical activity in hot climatic conditions. Int Z Angew Physiol 1969; 27(1): 43-50.

66. Costantini A, Nappo A, Pala MI, Zappone A. High dose thiamine improves fatigue in multiple sclerosis. BMJ Case Rep 2013; 2013.

67. Reynolds EH. Multiple sclerosis and vitamin B12 metabolism. J Neuroimmunol 1992; 40(2-3): 225-30.

68. Bitarafan S, Harirchian MH, Nafissi S, Sahraian MA, Tohgha M, Siassi F, et al. Dietary intake of nutrients and its correlation with fatigue in multiple sclerosis patients. Iran J Neurol 2014; 13(1): 28-32.

69. Yaya M, Ota K. Experimental and clinical studies on dysregulation of magnesium metabolism and the aetiopathogenesis of multiple sclerosis. Magnes Res 1992; 5(4): 295-302.

70. Cruciari R, Dvorkin E, Homel P, Culliney B, Malamud S, Shaiova L, et al. L-carnitine supplementation for the treatment of fatigue and depressed mood in cancer patients with carcinate deficiency: A preliminary analysis. Ann N Y Acad Sci 2004; 1033: 168-76.

71. Steen G, Axelsson B, Bowallius M, Holthus N, Molander BM. Isoprenoid biosynthesis in multiple sclerosis. Acta Neurol Scand 1985; 72(3): 328-35.

72. Sanoobar M, Dehghan P, Khalili M, Azimi A, Seifar F. Coenzyme Q10 as a treatment for fatigue and depression in multiple sclerosis patients: A double blind randomized clinical trial. Nutr Neurosci 2016; 19(3): 138-43.

73. Soleimani M, Jameie SB, Barati M, Mehrlizadeh M, Kordari M. Effects of coenzyme Q10 on the ratio of TH1/TH2 in experimental autoimmune encephalomyelitis model of multiple sclerosis in C57BL/6. Iran Biomed J 2014; 18(4): 203-11.

74. Sorgun MH, Yucelsan C, Tegin C. Is malnutrition a problem for multiple sclerosis patients? J Clin Neurosci 2014; 21(9): 1603-5.

75. Pooyandjoo M, Nouhi M, Shah-Bidar S, Djafarian K, Olyaeeemanesh A. The effect of (L-)carnitine on weight loss in adults: A systematic review and meta-analysis of randomized controlled trials. Obes Rev 2016; 17(10): 970-6.