Original Communication

Riboflavin Supplementation to Patients with Multiple Sclerosis does not Improve Disability Status nor is Riboflavin Supplementation Correlated to Homocysteine

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Abstract: Background: Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system. Riboflavin is involved in myelin formation in nerve cells. Riboflavin is a precursor of flavin adenine D-nucleotide (FAD), which is a coenzyme of methylene tetrahydrofolate reductase (MTHFR), which is an important enzyme for remethylation of homocysteine. Riboflavin supplementation has been shown to affect the serum levels of homocysteine in healthy volunteers. The aim of the present study was to test the effect of riboflavin supplementation on the status and disability of patients with MS and whether this effect could be mediated by serum homocysteine levels. Materials and Methods: This was a randomized, double-blind, controlled trial in which 29 MS patients with a mean age of 33 were tested with riboflavin, and the placebo group, with a mean age of 31, received either riboflavin supplementation (10 mg) or the placebo daily for six months. Disability, measured by the Expanded Disability Status Scale (EDSS) scores, erythrocyte glutathione reductase activity coefficient (EGRAC),

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and serum homocysteine levels were measured before and after the study. Results: The mean ± SD of EDSS score was significantly decreased in both groups over the six months of the study (2.3 ± 0.7 vs. 1.6 ± 0.6 for the riboflavin group and 2.8 ± 1.1 vs. 2.3 ± 1.3 for the placebo groups. The comparison across both groups yielded a non-significant change (P = 0.001 and 0.02, respectively). No significant differences were observed between the two groups in terms of EGRAC, riboflavin deficiency levels by EGRAC category, and serum homocysteine levels before and after the study. Conclusion: Riboflavin supplementation (10 mg/day) to patients with MS does not improve disability status. It appears that this effect is not related to serum homocysteine levels.

Key words: riboflavin; multiple sclerosis; EDSS; homocysteine

Introduction

Multiple sclerosis (MS) is a disorder characterized by a chronic demyelination and inflammation of the central nervous system [1]. MS is the most common non-traumatic cause of disability in the world [2]. It is estimated that more than 2.5 million people have MS worldwide, with approximately 25,000 people living with MS in Iran [2]. Progression in disability as measured by an increase in the Expanded Disability Status Scale (EDSS) is a frequently used outcome variable in clinical trials concerning MS. The EDSS has a possible range from 0, indicating no disability and normal neurological examination, to 10, referring to death due to MS [3]. One of the most significant current treatments of MS is pharmaceutical intervention. First-line, immune-modulating drugs such as beta interferon and glatiramer acetate, reduce relapse rates by about 30 % and can help preserve cognition. However, major problems with pharmaceutical intervention are the short- and long-term side effects of such drugs and whether they make a real difference to the progression of the illness [4].

Riboflavin is a water-soluble vitamin and is needed in the human diet [5]. Riboflavin acts as an antioxidant in the regulating processes of the nervous system. Also, this vitamin is involved in the metabolism of essential fatty acids in brain lipids, which play an important role in brain function. Studies in animals and humans have shown that riboflavin plays an important role in formation of myelin in both the central and periphe-ral nervous systems [6–9]. In a study designed for the evaluation of the association between nutritional factors and MS among incident cases and frequency matched controls, a significant protective effect was observed with riboflavin [7]. Myelin lipids, cerebrosides, sphingomyelin, and phosphatidylethanol amine (an important component of the myelin membrane) are significantly reduced in riboflavin deficiency. Thus, the effects of riboflavin deficiency are similar to fatty acid deficiency, causing rapid disruption of brain development and maturation [6].

Homocysteine is a vitamin B metabolite. This biomarker has been proposed to play several roles in MS pathogenesis [10]. Studies have shown that the plasma levels of riboflavin influence the plasma levels of homocysteine in alcohol-dependent patients and that the influence did not depend on its interaction with folate [11]. Also, riboflavin supplementation affected the serum levels of homocysteine in healthy volunteers [12, 13]. Dietary riboflavin supplementation during pregnancy has been shown to have an effect on the woman's homocysteine levels [14].

Riboflavin is a precursor of various flavin coenzymes, in particular flavin adenine D-nucleotide (FAD), which is a coenzyme of methylene tetrahydrofolate reductase (MTHFR). When FAD is substituted for MTHFR, it catalyzes 5, 10-methylenetetrafolate to 5-methylenetetrafolate, which acts as a donor of methyl groups for the remethylation of homocysteine [11].

To date, the research has tended to focus on prospective trials to determine whether the treatment with supplements and correcting the biomarker levels in the early stage of the disease can change the course of the disease [10]. The aims of this study were to evaluate the riboflavin status of MS patients, evaluate the effect of supplementation on the riboflavin status and the disability of patients with MS, and determine whether this effect could be mediated by serum homocysteine levels. Most studies related to the association between riboflavin and MS have focused only on the effects of riboflavin deficiency on the models of demyelinating neuropathy in animal studies [6, 8, 9] or the association between nutritional factors and MS in clinical patients [7]. However, no research has been found that surveyed the riboflavin status and the effect of riboflavin supplementation on disability in MS patients.

Subjects and Methods

Population, sampling and research design

This study was a randomized, double-blind controlled trial. Participants were MS patients under treatment at an outpatient clinic at Golestan Hospital at Ahvaz Jundishapur University of Medical Sciences (AJUMS) in Ahvaz, Iran, between September 2010 and May 2012. Inclusion criteria were: agreement to participate in the study, age 18–50 years, definitive diagnosis of MS using the McDonald criteria [15] based on history, physical examination, and laboratory tests, having relapsing-remitting MS (RRMS) or secondary progressive MS (SPMS), and EDSS ≤4 while exhibiting no disability, minimal signs in one functional system, or being fully ambulatory without aid. Exclusion criteria were: other types of MS [primary progressive multiple sclerosis (PPMS) and benign multiple sclerosis (BMS)], pregnancy, significant health conditions (e.g., coronary heart disease, uncontrolled diabetes mellitus, and liver disease), receiving other vitamins, participation in other clinical trials simultaneously, and having an EDSS score >4 while being ambulatory without aid, needing to rest after walking about 200 meters, or experiencing impairment of daily activities.

The study protocol was approved by the Medical Ethics Committee of AJUMS. The potentially eligible patients were defined by a neurologist, and they were invited to participate in this randomized, double-blind placebo-controlled trial.

Since available data on the effect of riboflavin supplementation on the development of MS were not sufficient for an exact statistical calculation of the appropriate sample size, the study was planned as a pilot study to recruit 30 patients for each study arm, i.e. a total of 60 patients. Fifty-four patients who met the inclusion criteria were included in the study. Eleven patients in the placebo (control) and 14 in the riboflavin groups (treatment) were lost to follow-up. Finally, 18 patients in the placebo and 11 patients in the riboflavin group completed the study (Figure 1). We used G*Power software (version 3.1.9) to calculate the study's power, and found it to be >80 % ($\alpha = 0.05$) to detect a difference in mean EDSS scores between groups.

All participants provided informed consent before entering the study. The participants were assigned randomly to riboflavin supplementation (treatment) or placebo (control) groups. The participants were allowed to continue their MS disease-modifying therapies. The duration of the study was six months of riboflavin supplementation [16] followed by a three-month "washout" period. The safety of the intervention was assessed monthly by a neurologist by recording adverse event reports and evaluating the patient's general condition. Participants in the riboflavin group received riboflavin capsules (10 mg/day) [17], and those in the placebo group received placebo capsules containing lactose; all participants took one capsule per day. Both capsules were prepared by the Pharmacy School of AJUMS. The dose that was used in this study is safe for the general population. But, due to a lack of suitable data, ULs (Tolerable Upper Intake Levels, the highest level of daily nutrient intake that is not likely to pose any risk of adverse health effects to almost all individuals in the general population) have not been established for riboflavin. The dietary reference intake (DRI) for riboflavin, which includes recommended dietary allowances (RDAs) and adequate intakes (Als), is 1.7 mg/day [5].

Fasting blood samples were collected at baseline and after the six-month study period to measure the nutritional status of riboflavin, homocysteine serum levels, and blood type to control as a possible confounder [18].

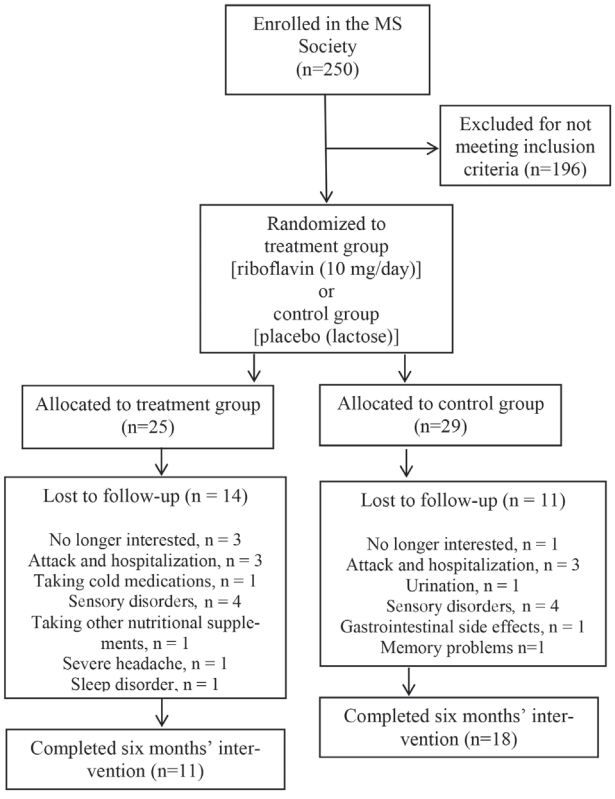

Figure 1: Flowchart of study design and subjects' participation throughout the study.

Blinding

The patients and the study's staff remained blinded to which patients were receiving riboflavin or the placebo throughout the entire treatment period of six months. Substantial efforts were made to maintain blinding, i.e. similar packages containing colored, opaque capsules were used for both the supplement and the placebo. All packages (including the placebo and the supplement) were labeled with a ready explanation on how to use the capsule (on an empty stomach), what to do if the patient forgot to take the capsule, and possible complications. For the double-blind method, a third person (an MS Society interface who knew about the allocations) filled the packets of the capsules (for one month's use), coded the capsule packets, and delivered them to the patients monthly. Patients were visited by the research physician for any adverse events, relapses, or side effects. All of the neurological examinations were performed by the same neurologist.

Primary outcome variables

The primary outcome measures were nutritional status of riboflavin and serum levels of homocysteine. Fasting blood samples were collected in potassium EDTA–containing tubes for riboflavin assessment or into tubes with no anticoagulants added for serum homocysteine concentration tests. Riboflavin status was assessed as the Erythrocyte Glutathione Reductase Activity Coefficient (EGRAC) using the Goldberg and Spooner method [19] by spectrometry set to 340 nm [20] (Glutathione Reductase kit; Randox, Britain). The cut-off points for determining riboflavin status were an EGRAC < 1.20 for normal riboflavin status (low risk), 1.20 ≤ EGRAC < 1.40 for marginally-deficient riboflavin status (medium risk), and EGRAC ≥ 1.40 for riboflavin deficiency [21].

The serum homocysteine concentration was assessed by the Axis® homocysteine enzyme immunoassay (EIA) method [22]. According to the interpretation of the serum homocysteine level results, patients were categorized as normohomocysteinemic with a fasting homocysteine < 35 μmol/liter and hyperhomocysteinemic with a fasting homocysteine ≥ 35 μmol/liter [23].

Secondary outcome variables

Disability was assessed by a neurologist at baseline and at the end of the study (after six months) as a secondary outcome measure, based on an ordinal scale ranging from 0 (normal) to 10.0 (dead). Patients were classified into three classes of disability based on the EDSS scores: 1) scores of 0.5–4.0 indicated mild impairment, and the patients were able to walk at least 500 meters without aid or rest; scores of 4.5–7.0 indicated increasing limitations in their ability to walk; and scores ≥ 7.5 indicated that the patients could not walk and had dysfunctions of their upper extremities [24].

Table I: Baseline demographic and clinical data of patients in both groups

Characteristic	Riboflavin (n = 11)	Placebo (n = 18)	P value[§]
Sex*			
Male	3 (27.3)	8 (44.4)	0.3
Age, y, mean (SD)[†]	8.7 ± 33.0	10.6 ± 30.6	0.2
Marital status*			
Married	(60)6	(62.5) 10	0.7
Education, n (%)*			
Primary	1 (12.5)	1 (7.1)	0.2
Secondary	1 (912.5)	7 (50)	
High school	(37.5) 3	3 (21.4)	
Diploma and higher	(37.5) 3	(21.4) 3	
Serum Hemoglobin[†]	1.6 ± 12.6	1.4 ± 12.6	0.1
Blood groups*			
O+	5 (45.5)	5 (27.8)	
A+	0 (0)	6 (33.3)	0.2
B+	4 (36.4)	5 (27.8)	
AB+	1 (9.1)	0 (0)	
O–	1 (9.1)	2 (11.1)	
EDSS[†]	2.3 ± 0.7	2.8 ± 1.1	0.2
EGRAC[†]	0.3 ± 1.1	1.2 ± 0.6	0.4
EGRAC category*			
Low risk (< 1.2)	10 (90.9)	17 (100)	0.4
Medium risk (1.2–1.4)	0 (0)	0 (0)	
Deficiency (> 1.4)	1 (9.1)	0 (0)	
Serum homocysteine levels (μmol/l)[†]	21.5 ± 6.6	21.6 ± 7.4	0.9
Hyperhomocysteinemic*	10 (90)	13 (72.2)	0.2
Normohomocysteinemic*	1 (9.1)	5 (27.8)	

*The results are expressed as number (percentage). The chi-square test was used to analyze data. [†]Results are expressed as mean ± SD. The independent sample T-test was used for analysis. [§]No significant differences were observed in terms of study variables between the two groups at baseline.

Analysis

Descriptive statistics were presented as mean ± SD for quantitative variables and as numbers (percentages) for qualitative variables. We examined the normality of the data by the Kolmogorov-Smirnov test. All data were normally distributed. In order to assess between-group variations in the riboflavin and placebo groups, various tests were conducted, including the independent sample *t*-test (to assess quantitative demographic variables and the differences in mean percent change in EDSS between groups at the end of six months), paired-samples *t*-test (to assess EDSS scores, EGRAC, and serum levels of homocysteine at baseline and at the six-month follow-up), chi-squared test (to compare the qualitative demographic variables, riboflavin efficiency rating by EGRAC category, and normohomocysteinemic and hyperhomocysteinemic patients, and a logistic regression test with the backward stepwise block method to determine and compare the odds ratios of normohomocysteinemia and hyperhomocysteinemia in the study groups after six months of supplementation. McNemar's test was conducted to determine the number of hyperhomocysteinemic patients who became normohomocysteinemic and the number of hyperhomocysteinemic patients who remained hyperhomocysteinemic after six months of supplementation. All statistical analyses were performed using SPSS version 21 (SPSS Inc., Chicago, IL). All tests were two-sided and p values < 0.05 were considered statistically significant.

Results

Fifty-four patients who met the inclusion criteria were included in the study. Of these, twenty-five patients were lost to follow-up and 29 patients completed the study. The reasons for the losses were self-reported by the patients (Figure 1).

Demographic and clinical characteristics of the patients

Table I provides the demographic and clinical characteristics of the patients who participated in the study. No significant differences were observed in terms of gender, age, marital status, education, and blood types between the two groups.

The effect of riboflavin supplementation on disability

The mean EDSS score was decreased significantly in the patients in both groups after six months of supplementation compared with baseline ($p = 0.001$ for patients taking riboflavin vs. $p = 0.02$ for patients taking the placebo). Also, the differences in mean percent change in EDSS between groups at the end of six months were examined using the independent sample *t*-test. The mean percent change in EDSS for the riboflavin group was 26.4 %, which was non-significantly smaller than the 15.4 % observed for the placebo group ($p = 0.25$). This finding could be due to the limited sample size of our study (Table II).

Table II: Participant Expanded Disability Status Scale (EDSS) at baseline and after a six- month follow-up and the differences in mean percent change in EDSS between groups at the end of six months*

Patients characteristics	Riboflavin (n = 11)		P value	Placebo (n = 18)		P value
	Baseline	After supplementation		Baseline	After supplementation	
EDSS score*	2.3 ± 0.7	1.6 ± 0.6	0.001	2.8 ± 1.1	2.3 ± 1.3	0.02
Percent change in EDSS**		26.4 %			15.4 %	0.25

* Results are expressed as mean ± SD. Six month changes within the riboflavin group, paired-samples t test was conducted for outcomes. The EDSS score mean was significantly decreased in the patients in both groups after six months of supplementation compared with baseline. ** The mean percent change in EDSS between groups at the end of six months supplementation. Differences have been showed using the Independent-samples t test.

Table III: The Comparison of Erythrocyte Glutathione Reductase Activity Coefficient (EGRAC), riboflavin deficiency rating assessed using EGRAC, and serum homocysteine levels between the two study groups before and after six months of supplementation

Variables	Riboflavin (n = 11)		Placebo (18 = n)		P value[§]
	Baseline	After supplementation	Baseline	After supplementation	
[†] EGRAC	0.3 ± 1.1	0.2 ± 1.2	1.2 ± 0.6	0.8 ± 1.1	0.9
EGRAC category *					
Low risk (< 1.2)	10 (90.9)	11 (100)	17 (100)	15 (83.3)	
Medium risk (1.2 – 1.4)	0 (0)	0 (0)	0 (0)	2 (11.1)	0.7
Deficiency (> 1.4)	1 (9.1)	0 (0)	0 (0)	1 (5.6)	
Serum homocysteine levels (μmol/l) [†]	21.5 ± 6.6	16.7 ± 3.6	21.6 ± 7.4	16.5 ± 7.6	0.07

* The results are presented as number (percentage). A chi - square test was conducted for data analysis. [†] Results are presented as mean ± SD. A paired T-test was conducted for data analysis. [§] No significant difference was observed between the two groups of patients in terms of EGRAC, riboflavin efficiency rating, and serum homocysteine levels before and after supplementation. After supplementation, the mean of serum homocysteine levels decreased in both groups. But it was not statistically significant.

The effect of riboflavin supplementation on EGRAC, riboflavin deficiency, and serum levels of homocysteine

Table III compares the erythrocyte glutathione reductase activity coefficient (EGRAC) to assess the nutritional status of riboflavin, riboflavin-deficiency rating measured with EGRAC category, and serum levels of homocysteine between the study groups before and after the supplementation period. No significant differences were observed between the two groups of patients in terms of EGRAC, riboflavin-deficiency rating, and serum levels of homocysteine before and after supplementation. After supplementation, the mean serum levels of homocysteine decreased in both groups, but the decreases were not significant.

The proportion of normo- and hyperhomocysteinemic subjects after riboflavin supplementation

Table IV compares the proportion of normohomocysteinemic and hyperhomocysteinemic subjects between the riboflavin and placebo groups and the odds ratios at baseline and after six months of supplementation.

Change in the prevalence of hyperhomocysteinemia at the six-month follow-up was also analyzed by McNemar's test. Five hyperhomocysteinemic patients in the riboflavin group and five in the placebo group were normohomocysteinemic after six months of supplementation. Also, five hyperhomocysteinemic patients in the riboflavin group and eight in the placebo group remained hyperhomocysteinemic after six months of supplementation. There were no significant differences between the two groups in terms of the proportion of the normohomocysteinemic and hyperhomocysteinemic subjects, and there was no difference in odds ratios for the hyperhomocysteinemia at baseline and after six months of supplementation.

Discussion

Several studies have been conducted on the effects of dietary interventions on MS patients [7, 16, 25] and on animal models of MS [26]. Mastronardi *et al.* showed that experimental autoimmune encephalomyelitis (EAE), an animal model of MS, improved with vitamin B12 supplementation [26]. However, the role of homocysteine in MS has not yet been well established [27].

To the best of our knowledge, this is the first study that has assessed the effect of riboflavin on disability in MS patients. This study addressed the issue of the effect of riboflavin supplementation on disability in MS patients and investigated whether this effect is related to homocysteine levels in the patients' blood. It was concluded that, although supplementation with riboflavin (10 mg/day) for six months reduced the EDSS score significantly in patients with MS, this reduction also occurred in the placebo group, and it was independent of serum homocysteine levels. The percent decrease in EDSS for the riboflavin group

Table IV: The comparison of the normohomocysteinemic and hyperhomocysteinemia* and Odds ratios** between the study groups after six months of supplementation

Characteristics	Riboflavin (n = 11)		(18 = n) Placebo		P value[§]
	Baseline	After supplementation	Baseline	After supplementation	
Hyperhomocysteinemic[†]	10 (90)	5 (62.5)	13 (72.2)	8 (47.1)	0.1
Normohomocysteinemic[†]	1 (9.1)	3 (37.5)	5 (27.8)	9 (52.9)	
Odds ratio (95% confidence interval)	6 (0.5–73.4)	1	1.6 (0.3–1.6)	0.53 (0.1–3)	0.6
Number of responses[α1]		5		5	
Number of not responses[α2]		5		8	

*The Chi-square test was used for statistical analysis. **Logistic regression test with the backward stepwise block method was used for statistical analysis. [†]The results are presented as a number (percentage). [α1] is the number of hyperhomocysteinemic patients who were normohomocysteinemic after 6 months of supplementation. [α2] is the number of hyperhomocysteinemic patients who remained hyperhomocysteinemic after six months of supplementation was conducted to analyze the data. Number less than the total number is because of the missing data. [§]There were not significantly different between the two groups in terms of the proportion of the normohomocysteinemic and hyperhomocysteinemic subjects and odds ratio at baseline and after six months of the supplementation.

was non-significantly greater than that of the placebo group. Several studies have shown that higher intake of riboflavin is negatively correlated with the risk of MS [7, 16, 28]. A Russian study showed a significant reduction in the neurogenic symptoms assessed with EDSS in the group that received cytoflavin, a drug that contains 2 mg of riboflavin as riboflavin mononucleotide (FMN), whereas such a reduction did not occur in the control group. The researchers concluded that the observed reduction in EDSS and improved cognitive function resulted from the decrease in the lipid peroxidation levels and content of myelin basic protein antibody [16]. Another study showed the protective effects of cytoflavin in terms of the normalization of energy metabolism, reduction of lipid peroxidation, and the reactivation of antioxidant systems in the spinal cords of rats with dense injury at the level of T-10 and T-11 [28].

We also measured the EGRAC to evaluate the nutritional status of riboflavin in patients with MS and its association with the disability measured by EDSS. The results showed no significant differences between the two groups in terms of EGRAC and riboflavin-deficiency rating evaluated with the EGRAC category before and after supplementation. Based on a literature review, no study has assessed the nutritional status of riboflavin in patients with MS; therefore, it is not possible to compare these findings with the results of similar studies.

This study evaluated the plasma levels of homocysteine in patients with MS and their relationship with the clinical disability of these patients. The results showed that, at baseline, the mean level of homocysteine of both groups, i.e. 21.5 μmol/L, decreased in both groups after supplementation for six months, but the decreases were not statistically significant. Also, 72.2 % of the patients who received the placebo and 90 % of the patients who received riboflavin supplements were hyperhomocysteinemic at baseline, but those percentages were reduced to 47.1 % and 62.5 %, respectively, after six months of supplementation. Although the rate of decrease was slightly higher in the riboflavin group than in the placebo group, this difference was not statistically significant. McNemar's test was used to determine the number of hyperhomocysteinemic patients who did not respond to the six-month treatment with supplementation, i.e. they remained hyperhomocysteinemic. The results showed that the number of patients who remained hyperhomocysteinemic after six months of follow-up was greater in the placebo than in the riboflavin group (8 vs. 5, respectively). However, the mean of homocysteine levels at baseline (21.5 μmol/L) in this study was significantly greater than the results of a recent study [27]. A meta-analysis to assess the association between homocysteine serum levels, folate, vitamin A, and vitamin B12 in patients with MS showed that they have elevated homocysteine levels and low levels of vitamin B12 [29].

A similar study to assess the plasma levels of homocysteine in patients with MS showed a significant increase compared to controls in the absence of significant differences in the serum levels of folate and vitamin B12 [10].

Elevated homocysteine levels can cause several neurotoxic activities and cardiovascular dysfunction that may be involved in the pathogenesis and progression of MS [27, 30].

The results of this study indicated that, although the daily riboflavin supplementation with a dose of 10 mg of riboflavin for six months could significantly reduce the EDSS score in MS patients, it seems the mechanism of riboflavin in disability improvement of these patients is independent of serum homocysteine. Since a previous study in Russia indicated that the reduction of lipid peroxidation levels and the content of myelin basic protein antibody may be the cause of cytoflavin's inducing improvement in MS patients' disability, it is necessary in future studies to determine the exact mechanism of riboflavin's effect on the improvement of disability in patients with MS by evaluating the immunity indices and measuring the levels of anti-myelin antibodies involved in the pathogenesis of MS [31], and their association with the riboflavin status and supplementation.

The patients were allowed to leave the study at any time they wished, and the dropout rate of this study was high (56 % withdrew from the riboflavin group and 38 % withdrew from the control group). The dose used in this study was safe [5], and the high drop-out rate may have been due to the absence of an established UL for riboflavin. The reasons we have indicated for the high drop-out rates were obtained from self-reporting of the patients who withdrew. The patients may have attributed their common side effects of disease, such as difficulty with urination and sleep disturbance, to the wrong intervention, thus they tended to withdraw from the study.

The power of this study was that we reported the probable effect of riboflavin supplementation on disability improvement of patients with MS for the first time. Also, we assessed the nutritional status of riboflavin in this disease. The limitation of our study was that the clinical implications of our finding are not entirely clear, since MS patients have shown elevated serum homocysteine levels in previous studies [10].

Also, we could not find any association between serum levels of homocysteine and the disability index measured in this study (EDSS) in the subgroup of patients with MS. In addition, since the patients in both groups (riboflavin and placebo groups) showed a decrease in the mean EDSS score at the six-month follow-up, it is currently unknown whether riboflavin supplementation treatment is the optimal way to treat this subgroup of patients with MS, however these results can provide a basis for future studies.

Conclusion

It was concluded that supplementation with riboflavin (10 mg/day) for six months reduced non-significantly the EDSS mean by 26.4 % vs. 15.4 % for MS patients taking the placebo, and that this difference was not associated with the serum levels of homocysteine.

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