Multiple sclerosis (MS) is a relatively common disease with unknown etiology and no cure which results in neurological disability in young adults. This condition affects over two million people worldwide¹ and over 60,000 individuals in Iran². Many of the current treatments are costly, limited in efficacy, and possess unpleasant side effects³. Although the exact etiology of developing MS is dependent on both genetic and environmental factors⁴, pathological events such as impairment of T helpers (Th) are involved⁵. The major types of Th cells are Th1 cells that produce interferon-γ (IFN-γ), Th2 cells that produce interleukin-4 (IL-4)⁶,⁷ and Th1/Th2 balance is considered one of the risk factors in MS etiology⁸,⁹,¹⁰. IFN-β treatment shifts the immune

Regulation of Lipid-dependent Membrane Enzymes by Hot Nature Diet with Co-supplemented Hemp Seed, Evening Primrose Oils Intervention in Multiple Sclerosis Patients

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Multiple sclerosis (MS) is the most chronic and inflammatory disorder that result in physical disability. Because of limited efficacy and adverse side effects, identifying novel therapeutic and protective agents is important. This study was aimed to assess regulation of surface-membrane enzymes by hemp seed and evening primrose oils as well as Hot nature dietary intervention in Relapsing Remitting MS (RRMS) patients. In this double blind, randomized trial, 100 RRMS patients with EDSS<6 were allocated into 3 groups: "Group A" who received co-supplemented hemp seed and evening primrose oils with advising Hot nature diet, "Group B" who received olive oil as placebo, "Group C" who received the co-supplemented oils. Clinically EDSS and functional score as well as biochemical parameters (blood cells PUFA, FADS2, Serum sPLA2) were assessed at baseline and after 6 months. Mean follow-up was 180±2.9SD days (N=65, 23 M and 42 F aged 34.25±8.07 years with disease duration 6.80±4.33 years). There was no significant difference in studies parameters at baseline. After 6 months, significant improvements in EDSS and functional score were found in the group A and C while EDSS and pyramidal score showed significant increase in group B. Biochemical parameters showed improvement in the A and C groups whereas there was worsening condition for the group B after the intervention. The co-supplemented hemp seed and evening primrose oils with Hot nature diet can have beneficial effects in improving clinical symptoms and signs in RRMS patients which were confirmed by regulation of surface-membrane enzymes.

Key words: Relapsing Remitting Multiple sclerosis, Hot nature diet, Evening primrose (Oenothera biennis L), Hemp seed (Cannabis sativa L), PUFA, Delta-6-desaturase (FADS2), Phospholipase A2.

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response from the Th1 to Th2 pattern by enhancing the production of anti-inflammatory Th2 cytokines (ex. IL-4) and decreasing the production of pro-inflammatory Th1 cytokines (ex. IFN-γ).

Traditional Iranian Medicine (TIM) practiced in Iran and Cold and Hot natures (Mizadj) have been believed to exist in TIM and in many other traditional medical theories. The study of Shahabi et al, on IL-4 / IFN-γ ratio showed that the tendency of the Hot nature people was to deviate toward Th2-like immune responses to a greater extent than of the Cold-nature people. In this way, consumption of Hot-nature foods in a person suffering from an autoimmune disease with a deviation toward Th1 immune responses (such as MS) may be useful because they can accelerate warmth of nature and deviation toward Th2 immune responses. Evidence was found that omega3-polyunsaturated fatty acids (ω3-PUFAs), can suppress IFN-γ production in MS patients, metabolism of PUFA is controlled by Phospholipase-A2 (PLA2) appears to play a fundamental role in cell injury in the central nervous system (CNS) as well as in the pathogenesis of MS-like and production of pro-inflammatory mediators. The PLA2 hydrolyzes phospholipids to release arachidonic acid (AA) which can mediate inflammation and demyelination, hallmarks of the CNS autoimmune disease MS. A study showed that PLA2 concentration increased 6-fold in the urine of MS patients with active disease and 4-fold in patients in remission, regardless of immune-modulating therapy. We supposed that combination of hemp seed oil (HSO) and evening primrose oil (EPO) with Hot nature dietary and the co-supplemented oils can affect membrane phospholipids fatty acid composition, with increase cell membrane PUFAs, also effects of intervention appear to possess anti-inflammatory roles; and inhibit increase in pro-inflammatory cytokines and PLA2, and may represent novel therapeutic strategies against MS. This study is designed to evaluate effects of co-supplemented oils with Hot nature diet on clinical and biochemical parameters of RRMS patients.

**MATERIALS AND METHODS**

This double-blind, randomized clinical trial was carried out on 100 RRMS patients to determine the therapeutic and protective effects of Hot nature dietary and the co-supplemented oils. The study was approved by the Neurosciences Research Center (NSRC) and local Ethics Committee of Tabriz University of Medical Sciences. MS patients were contacted and recruited through the MS Society of Tabriz. Patients with a definite diagnosis of MS using the Kurtzke Extended disability status score (EDSS) <6 criteria; with relapsing-remitting type of MS (RRMS); ages 14-55 years were enrolled. Patients with secondary or primary progressive MS, pregnancy, corticosteroid treatment, patients suffered concomitantly from another chronic disease such as rheumatic diseases, serious heart diseases, malignant tumors, and other neurological and inflammatory illnesses were excluded. Patients were allowed to continue their routine medications. A written informed consent was completed prior to the study for all patients. The patients completed a 3-d food record in the first week, a non-quantitative Food Frequency Questionnaires (nqFFQ) to assess food and drinks consumed and dietary habits. They were asked to maintain their usual level of physical activity and not to consume any supplements during the study. The patients were then randomly assigned to receive three dietary interventions:

- **Group A** Those receiving the co-supplemented oils, 18-21g/day (6-7g, three times daily) with advising Hot nature diet,
- **Group B** Those consuming olive oil 18-21g/
day (6-7g, three times daily),

Group C: Those receiving the co-supplemented oils, 18-21g/day (6-7g, three times daily) for 6 months.

To achieve this objective, group A was asked to consume "Hot nature diet" with a wide choice of foods and drinks items permitted during each dietary period and delivered at home for 6 months (Appendix A). Groups B and C were asked to consume their usual diet during the intervention. "Hot nature diet" includes foods with Hot nature, low intake of cholesterol, hydrogenated or trans fatty acids and saturated fats (fried foods), consumption of olive or grape seed oils as main oils in diet, eating plenty of fresh fruit and vegetables with Hot nature, nuts and seeds without additives, fish and seafood, unrefined carbohydrates, drinking plenty of water (avoiding too much drink containing artificial additives, sweeteners or other stimulants), cutting down sugar and refined starch (i.e. non-whole meal bread, cakes, pastries, biscuits, sweets and soft drinks), consumption of dairy products with honey or date and removing foods with Cold nature (Appendix B), avoid of alcohol and smoking. The patients were contacted monthly by telephone to assess compliance. After baseline assessments, 100 patients randomized to three groups according to following diagram (Fig. 1).

Fig 1. Flowchart of the study; 100 patients randomized to three groups; group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary; group B: Olive oil; group C: Co-supplemented hemp seed and evening primrose oils.

All measures were repeated similarly with same approach and assessors at the end of intervention period.

**Measurement of the disability status of patients**

A medical history to check clinical status (inc. EDSS and functional score) and medications used were assessed. The functional disability status (disease severity) of each patient was measured by a trained clinician using the Kurtzke EDSS. The EDSS quantifies disability in eight functional systems (FS) and allows neurologists to assign a functional system score (FSS) in each of them. The functional systems are pyramidal, cerebella, brainstem, sensory, bowel and bladder, visual, cerebral and "other". Scales for the total Kurtzke EDSS are from 0 to 10, in which the 0 score indicates no disability at all and 10 indicates death due to MS. Patients' Natures and Temperaments (Mizadj) was determined according to TIM by using a standard questionnaire. The Warmth/Coldness ratio was calculated for all patients based on the results obtained by the questionnaire.

**Blood sample processing and analysis**

Venous blood samples (10 ml) were collected from the patients before and 6 months after treatment. The red blood cells (RBCs) were washed in a 0.85% saline solution and immediately transferred to small glass vials, layered with nitrogen, and stored up to one year at -80°C. Serum was separated and aliquots were stored at -80°C. Total lipids were extracted from RBCs with chloroform/methanol (1:2 v/v), then fatty acids were separated from their alcohols and etherified by methanolysis to form fatty acid methyl esters (FAME). FAME were injected in gas chromatography and analysis composition to assess long-chain PUFAs. Serum FADS2 (D6D) and PLA2 levels from serum were measured by enzyme-linked immunosorbent assay (ELISA) (Uscn Life Science Inc). The absorbance of D6D, PLA2 levels was read at 450 nm. Percentages of PUFAs were measured against an internal standard. RBC membrane PUFAs as µg FA/ml packed RBC analyzed.

**Statistical analysis**

The Statistical analysis was performed using SPSS software (ver 14.0; SPSS Inc, Chicago, IL). Data was expressed as mean ± standard deviation (SD). Differences in clinical and biochemical variables between pre- and post within each intervention group were analyzed using paired t-test. Statistical significance was defined as p < 0.05.

**RESULTS**

**Clinical and biochemical results in RRMS patients**

One hundred (34 M and 66 F) patients were enrolled in this study. Figure 1 summarizes the patient attrition patterns in the study. The dropout rate was 35 from 100 patients (11 in group A, 11 in group B, and 13 in group C). This study was performed during between October 2010 and October 2011. The patients' characteristics and
demographics are shown in (Table 1). The sample consisted of 23 males and 42 females with a mean age of 34.25±8.07 years and mean disease duration of 6.80±4.33 years. There was statistically no significant difference in the mean age, gender, disease duration, interferon intake, and average age at onset between three groups.

Clinical and biochemical results

The clinical results of the trial are summarized in (Table 2). There were significantly

| Table 1. Clinical and demographic characteristics of the study patients n=65 (23 men, 42 women) |
| Variable | Group A (N=23) | Group B (N=22) | Group C (N=20) |
| Mean ± SD | Mean ± SD | Mean ± SD |
| Age(years)* | 34.2±7.5 | 35.9±7.8 | 33.7±7.8 |
| Average age at onset (years)* | 25.0±7.5 | 30.3±8.1 | 27.6±6.4 |
| Disease duration (years)* | 6.26±3.9 | 7.55±5.08 | 6.60±4.0 |
| Interferon intake | N (%) | N (%) | N (%) |
| Gender (M/F) | 16/7 | 11/11 | 15/5 |

Group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary
Group B: Olive oil
Group C: Co-supplemented hemp seed and evening primrose oils.

| Table 2. Effect of intervention on mean (±SD) clinical variables: Mizadj (Warmth/Coldness ratio), Expanded disability status score (EDSS) and relapse rate in trial groups of RRMS patients comparison to baseline |
| Variables | Group A (N=23) | Group B (N=22) | Group B (N=20) |
| Baseline | 6 months | P | Baseline | 6 months | P | Baseline | 6 months | P |
| Mizadj | 1.05±.63 | 1.74±.88 | 0.001 | .90±.47 | .78±.43 | 0.116 | 1.01±.63 | 1.22±.8 | 0.017 |
| EDSS | 2.76±1.39 | 1.77±1.7 | 0.001 | 3.45±1.41 | 1.41±3.86 | 0.005 | 3.25±1.94 | 1.83±2.95 | 0.002 |

* P for paired-t test
Group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary
Group B: Olive oil
Group C: Co-supplemented hemp seed and evening primrose oils.

| Table 3. Effect of intervention on mean (±SD) functional score in trial groups of RRMS patients comparison to baseline |
| Variables | Group A (N=23) | Group B (N=22) | Group C (N=20) |
| Baseline | 6 months | P | Baseline | 6 months | P | Baseline | 6 months | P |
| Pyramidal | .69±1.22 | .61±.98 | .328 | 1.32±1.25 | 1.68±1.12 | .029 | 1.15±1.53 | 1.25±1.41 | .494 |
| Cerebella | .74±1.0 | .52±.95 | .233 | 1.04±1.13 | 1.14±1.08 | .680 | .95±.94 | .70±1.03 | .171 |
| Brainstem | .57±.89 | .39±.72 | .103 | .68±.99 | .81±1.03 | .162 | .55±.10 | .40±.88 | .186 |
| Sensory | .57±.89 | .30±.70 | .186 | .91±.34 | .91±1.11 | 1.00 | .80±.10 | .70±1.22 | .606 |
| Bowel/Bladder | 1±1.31 | .35±.57 | .008 | 1.68±1.55 | 1.27±1.49 | .071 | 1.21±1.36 | 1.25±1.37 | .666 |
| Visual | .96±1.29 | .69±1.14 | .208 | 1.32±1.70 | 1.45±1.50 | .633 | 1.20±1.58 | 1.15±1.66 | .748 |
| Cerebral | .87±1.01 | .43±.66 | .009 | 1.05±.99 | .82±1.01 | .381 | 1.30±1.17 | 1.15±.93 | .330 |
| other Functional | .65±.49 | .35±.49 | .005 | .86±.35 | .73±.46 | .186 | .80±.41 | .40±.50 | .002 |

* P for paired-t test
Group A: Co-supplemented hemp seed and evening primrose oils and advising Hot nature dietary
Group B: Olive oil
Group C: Co-supplemented hemp seed and evening primrose oils.
better changes in Mizadj and EDSS in group A and C at the end of the intervention, while olive oil consumption resulted in a significant increase in EDSS. Based on results of (Table 3) globally all functions in groups A and C shows relating improving trend but only, there are statistically significant reduction in bowel/bladder, cerebral and "other functions" in group A and "other functional" scores in group C after 6 month intervention, whereas in group B there is relating trend to worsening in all functions, but only pyramidal function shows statistically significant increase. This results (Tables 2, 3) means that the co-supplemented oils with or without Hot nature diet used in our study might have a therapeutic effect towards MS. (Table 4) indicates that patients (baseline) have elevated serum level of PLA2 activities which might be due to increase
hydrolysis of membrane phospholipids by PLA2 is a well-known early response to tissue damage in all organ systems including CNS. Mean of D6D, PLA2 and PUFAs concentrations were significantly different before and after consumption of the co-supplemented oils with or without Hot nature diet in groups A and C, respectively. Red blood cells PUFAs rate showed significant increase, while D6D and PLA2 concentrations decreased significantly in groups A and C. No significant changes were found in PUFAs, D6D and PLA2 in group B (Table 4). This result suggests that the observed reduction of D6D was a consequence of the well-described effects of this type of intervention and increase of PUFAs and reduction in expression of PLA2 key enzymes, caused a decrease in mean EDSS. Surprisingly, altering PUFA rate caused a decrease in PLA2 expression, in particular, in the co-supplemented oils and Hot nature diet group.

**DISCUSSION**

**Possible mechanisms**

The theory of "Hot and Cold natures" founds its origin from ancient Greece medicine by Hippocrates (Greek physician, 460-375 BC) and Galen (199-129 BC). Hippocrates says let's our diet be our medicine, and Avicenna said that for each person there is specific foods for himself. The most important rule of all the ancient theories was the maintenance of the balance between the fundamental body elements, among which Warmth and Coldness played a completely essential role. To determine a persons' Warmth/Coldness (W/C) or Mizadj, it is observed that intermediate forms or combinations of two or more temperaments, so most people are under influence of both the Hot and the Cold elements and we could evaluate the severity of each nature in a person by W/C ratio (Table 2). It means that in a person with a very Hot nature, W/C ratio is high (such as allergic patients with tendency to Th2-like responses) and in a person with a very Cold nature, W/C ratio is low (such as MS patients with tendency to Th1-like responses). Therefore, an allergen can induce allergic reaction in Hot nature persons with a higher than in cold nature persons, because the former have a greater tendency to Th2-like responses. Based on studies, immune responses during infancy and early childhood are dominated by Th2 cytokines, but the shifting toward Th2 pattern decreases with age, and an allergen can induce allergic reaction in child with a higher strength than adults. This is in agreement with TIM's belief that the nature is dominated by Warmth at birth but its Warmth decreases with age. This critical point that we should indicate why MS attacks is observed in the start of adulthood age. Shahabi et al., showed the persons of a Hot nature had more deviation of the immune system toward Th2 responses than the persons of a Cold nature, and in agreement with TIM practitioners' view that MS (Th1-mediated autoimmune disease), is more prevalent in Cold nature persons than in Hot nature. This intervention is in agreement with the complications relating to Hot or Cold nature dominance and targets this key mechanism of disease and works like approved treatments. It may explain why therapies that promote a Th1 to Th2 cytokine-shift are beneficial in MS patients.

All approved therapies, besides many of those under investigation appear to possess immunomodulatory and anti-inflammatory roles as the main mechanism of action that Beta-interferons and Glatiramer acetate are on top of this list. Based on TIM practitioners' view that Hot nature foods is useful for MS patients, while Cold nature foods aggravates their disease, also women is dominated twice more than men by Cold nature and this confirms autoimmune diseases such as MS is mostly common in women more than men, parameters like weather coldness, lack of sun light exposure and stressful life enhance Coldness in subjects. Increase of W/C ratio (Mizadj) agreeing with increase of EDSS and functional score parameters were significantly better in groups A and C compared to group B, and case groups felt physically and emotionally healthier (Tables 2, 3). A trend favoring in group A was maintained on EDSS and functional score until the end of the study for all measurements while, no therapy exists that can confer prolonged remission in MS and therapeutic agents are only partly effective. Their long-term beneficial effects are uncertain with side effects. Agreeing with above changes, dietary PUFA(co-supplemented oils) and their metabolites affect inflammatory functions and cytokines production during the 6 months, because ω3-
PUFAs, can suppress IFN-γ production in MS patients, prior studies have demonstrated a relation between MS mortality and dietary fat, and lipids can be found in two structural components; the neuronal membrane (about 50%) and the myelin sheath (about 70%) and a high proportion of lipid 70-85% and the Blood-Brain Barrier (BBB) is a key to the bioavailability of brain essential fatty acid (EFA). Using chromatographic lipid profiling; we confirmed a significant increased in red blood cells PUFAs rate in groups A and C while in group B is no significant (Table 4).

The study showed that daily administration of the co-supplemented oils has beneficially affected the PUFAs of total cell lipids extracted from blood cell phospholipids. In addition, the PUFAs rate was correlated with the EDSS and functional score benefits at the last visit. This results likely due to remyelination that occurs during the early phases of disease; whereas this is rare at more progressed stages. Current estimates of the ω3/ω6 PUFAs ratio in developed countries are as low as 1:25 with recommendations to the public that it should be much higher (ideally 1:4). The ω6/ω3 ratio in HSO is normally between 2:1 and 3:1, which is considered optimal for human health. Horrobin showed that preliminary results the use of EPO and colchicine combined therapy in MS patients suggest that may be of considerable value. EPO is being used in increasing amounts in nutritional and pharmaceutical preparations, and there are claims that it may alleviate various chronic disease states. The EPO content of 9% GLA is the single most important parameter that is metabolized into DGLA, the natural precursor of PGE. β-Carotene is a pro-vitamin A and gives a characteristic color to EPO. The HSO / EPO ratio in this study is 9:1, so ω3/ω6 PUFAs ratio reach to 1:4 or higher, that is competitive inhibition of the conversion of dihomo-gamma-linolenic acid (DGLA) to arachidonic acid (AA) resulting in more anti-inflammatory prostaglandin E1 (PGE1). AA is a precursor of pro-inflammatory and pro-aggregator (PGE2), while gamma-linolenic acid (GLA) and DGLA are precursors of anti-inflammatory PGE1 (Appendix E). GLA is produced in the body from desaturation of linolenic acid (LA) by the reaction catalyzed by enzyme delta-6-desaturase (D6D or FADS2). D6D is the rate-limiting step in the PUFAs biosynthetic pathways that are incorporated into cell membranes, which thereby affect permeability, and functional properties of cells. The D6D deletion may prevent the conversion of alphalinolenic acid (ALA) into very long chain-PUFAs. Based on studies, the activity of D6D, which had become impaired by: aging, diabetes, viral infection, high alcohol intake, high level cholesterol, high blood pressure, radiation, stress-related hormones, deficiencies of zinc, magnesium, biotin, vitamins C, B6, B3 and excessive level of trans fatty acid, genetic deficient (inactive D5D and D6D enzymes). Moreover, excessive consumption of GLA occurs in: high rates of cell division, inflammatory, antiviral reaction and Trauma.

The noticeable presence of both GLA and stearidonic acid (SDA) in HS and EP oils, typically at a favorable ω6/ω3 ratio of 2:1 allows this enzymatic step with D6D to be efficiently bypassed. Because the produced result of this enzyme (GLA and SDA) is delivered to the patient organism by the co-supplemented oils in this trial, in this way, D6D concentration decreased significantly in groups A and C, while group B showed a non significant (Table 4). These mentioned basic parameters for cellular metabolic pathways could easily replace to this intervention in groups A and C. It is a strong reason for importance of the effects of diet and various nutrients for modulation in developed wrong disordered metabolic interaction in the metabolism of MS patients. In considering the inflammatory role of secretory PLA2 (sPLAs), phospholipids constitute approximately 40%, 60% and 90% of the total lipids in myelin, erythrocyte and mitochondrial, respectively, that play a role in double bio-membrane structure. Metabolism of PUFA in membrane phospholipids is controlled by PLA2 and acyltransferases known as the "deacylation-reacylation cycle". Evidence showed that sPLA2 involvement in diverse inflammatory conditions, implicating almost all of membranes in any organ of the body (such as myelin, erythrocyte and mitochondrial) and the Blood-Brain Barrier is a key to the bioavailability of brain essential fatty acid (EFA). Using chromatographic lipid profiling; we confirmed a significant increased in red blood cells PUFAs rate in groups A and C while in group B is no significant (Table 4).
peroxidation and mitochondrial damage, and may contribute to both acute forms of apoptosis and delayed inflammation induced tissue degeneration, finally demyelination that hallmarks of the CNS autoimmune disease MS. Mean levels of sPLA2 were increased 6-fold in the urine of MS patients with active disease and 4-fold for patients in remission, regardless of immune-modulating therapy. For this reason, PLA2 appears to play a fundamental role in cell injury in the CNS, and plays a key role in the pathogenesis of MS-like with production of pro-inflammatory mediators. However, up till now there are no effective sPLA2 or cPLA2 inhibitors available for clinical use, but extracellular PLA2 inhibitors suppress CNS inflammation. In this way, inhibition of specific PLA2 and elevated levels of inflammatory cytokines may represent novel therapeutic strategies against MS. We found that elevated serum of PLA2 activity in the patients (baseline) which is a well-known early response to tissue damage in all organ systems including erythrocyte, myelin in CNS and mitochondrial, etc. After study, PLA2 concentration decreased significantly in groups A and C and estimated PLA2 and D6D were both inversely correlated with PUFAs and these parameters in B group showed a non significant (Table 4).

The above findings imply that compared to olive oil, the co-supplemented oils with Hot nature diet produced a significant reduction in clinical symptoms and signs, and the patients general health and well-being improved that due to evidences on base higher PUFAs in peripheral tissue (red blood cells) and maybe in brain tissue and mitochondrial, etc. After study, PLA2 concentration decreased significantly in groups A and C and estimated PLA2 and D6D were both inversely correlated with PUFAs and these parameters in B group showed a non significant (Table 4).

Regarding the beneficial properties of this intervention, effects co-supplemented oils and Hot nature diet appear to possess anti-inflammatory roles; and have prophylactic and therapeutic properties in improving clinical symptoms and signs in RRMS patients which were confirmed by regulation of surface-membrane enzymes. Confirmation of importance of Warmth or Coldness of foods was advocated by many traditional medical theories, maybe one of the other conclusions of the present study.

**CONCLUSION**

Conclusions of this study demonstrated that the intervention had a significant clinical impact on RRMS patients in comparison with placebo, which was probably due to the high PUFAs content and antioxidant properties of co-supplemented oil supplements. This intervention was found to improve clinical symptoms and signs in RRMS patients in comparison with placebo. Overall, these findings support the hypothesis of EFA abnormalities in MS patients, and indicate that the problem could well be one of conversion of EFA to LC-PUFA (according to: appendix C), as originally suggested before. It is important to note that supplementation with PUFAs may require additional vitamin E intake to prevent increased peroxidation of membrane lipids, while the total amount of tocopherols of (α-, β-, γ-tocopherol, δ-tocopherol) HSO is high between 80 and 110 mg/100 g, with γ-tocopherol as the main tocopherol (85%) that exhibit potent antioxidative properties for scavenging free radicals. We must notice that the co-supplemented oils are foodstuffs and do not act as rapidly as most medications, so any effects will take time to appear. It means, most patients who respond to supplementation usually report noticeable benefits within one or two months. The minimum trial period should be at least six months, as studies have shown that it takes 10-12 weeks for PUFA levels in brain cell membranes to return to normal levels after a long-standing deficiency. In this intervention, minor adverse events was rare and much less than for medication commonly prescribed for RRMS patients and on recommendation, the co-supplemented oils might be given alone or during treatment with synthetic drugs to permit reduction of dose level of the later, and can administer orally.

**LIMITATIONS**

The limited duration of the intervention caused by budget limitation and consumption of the Co-supplemented oils as syrup is the other important one. So we would not able to encapsulate the supplement for patients' consumption by a protective coating or membrane, the high prevalence of dropout in this study is caused by the mentioned parameters. Uncontrolled diet is the other important confounding factor.

**FUTURE DIRECTIONS**

1. Based on studies the use of co-supplemented hemp seed and evening primrose oils (8:2 ratio is better, which is considered to be optimal for human health) either alone or in conjunction of different immunomodulation therapy synthetic drugs (which may have synergistic effects with each other) for longer periods in MS patients.

2. Provide a rationale for performing additional functional studies on the D-6-desaturase (FADS1) and D-5-desaturase (FADS2) gene transcription in all groups of MS patients and healthy adults, because, a strong correlation...
between MS and a rapid fall of delta-6-desaturase activity has been shown after intervention.

3. Hot nature diet and Supplementing with hemp seed and evening primrose oils may prevent several inflammatory diseases and neurodevelopmental and neurodegenerative disorders, because the changes in lipid biology identified in MS may be relevant to other psychiatric conditions.

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