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Association of Expanded Disability Status Scale and Cytokines after Intervention with Co-supplemented Hemp Seed, Evening Primrose Oils and Hot-natured Diet in Multiple Sclerosis Patients♦

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ABSTRACT

Introduction: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Because of limited efficacy and adverse side effects, identifying novel therapeutic and protective agents is important. The aim of this study is to examine the correlations between expanded disability status scale (EDSS) and cytokines after intervention with co-supplemented hemp seed and evening primrose oils and hot-natured diet in patients with relapsing-remitting multiple sclerosis (RRMS). ***Methods:*** We studied a group of 23 patients with clinically definite RRMS, with EDSS<6 who received co-supplemented hemp seed and evening primrose oils with advising hot-natured diet. Clinically EDSS and immunological factors (plasma cytokines of IL-4, IFN-γ and IL-17) were assessed at baseline and after 6 months. ***Results:*** Mean follow-up was 180±2.9 days (N=23, 7 Male and 16 Females aged 25.0±7.5 years with disease duration 6.26±3.9 years). After 6 months, significant improvements in extended disability status score were found in the patients in agreement with decrease cytokines of IFN-γ and IL-17 and increase cytokines of IL-4. Clinical and immunological parameters showed improvement in the patients after the intervention. ***Conclusion:*** Our study shows that co-supplemented hemp seed and evening primrose oils with hot-natured diet can have beneficial effects in improving clinical symptoms in relapsing remitting MS patients and significant correlation was found between EDSS and immunological findings.

Introduction

Multiple sclerosis is an immune-mediated disorder of the central nervous system[1] with unknown etiology and no cure which results in neurological disability in young adults. Since inflammation contributes to it, neurodegeneration is the major pathological correlate of clinical disability.[2] Drug therapy is often necessary.[3] Evidence supports that inflammation directly causes demyelination and pathological events such as impairment of T helpers (Th) are involved in it (inflammatory cascade).[4] The major types of Th cells are Th1 cells that produce interferon-*gamma* (IFN-*γ*), Th2 cells that produce interleukin-4 (IL-4)[5,6] and Th1/Th2 imbalance is considered one of the risk factors in MS etiology. In addition, Th17 (new T-cell subset) produces IL-17 cytokine (a key Player in MS pathogenesis) and cytokines derived from Th1 (IFN-*γ*) and Th2 cells (IL-4) are shown to repress the development of Th17 cells.[7-9] IFN-β treatment shifts the immune 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*). The Traditional Iranian Medicine (TIM) practiced in Iran and cold and hot natures (*Mizadj*) is believed to exist in TIM and in many other traditional medical theories.[10] The study of Shahabi *et al.* on IL-4 / IFN-*γ* ratio showed that tendency of hot-natured people deviated towards Th2-like immune responses to a greater extent than of the

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cold-natured people.[11] In this way, consumption of hot-nature foods in a person suffering from an autoimmune disease with a deviation towards Th1 immune responses (such as MS) may be useful because they can accelerate warmth of nature and deviation towards Th2 immune responses. Also, evidence was found that omega3-polyunsaturated fatty acids (ω3-PUFAs), can suppress IFN-γ production in MS patients[12] that 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. We supposed that combination of hemp seed (*Cannabis sativa L.*) and evening primrose (*Oenothera biennis L.*) oils, as co-supplemented oils, with hot-natured diet may reduce the pro-inflammatory cytokines and target this key mechanism of disease and work like approved treatments. Hemp seed has been used as a food/medicine in China at least 3000 years ago.[13] It contains over 80% PUFAs, with ω3/ω6 ratio between 1:2 and 1:3, which is optimal for human health.[14] Hemp seed oil contains phytosterols, terpenes and kinds of tocopherol that not only exhibits potent antioxidative properties for scavenging free radicals, but may also act on specific signaling pathways for regulating inflammatory responses.[15-18] By the presence of gamma linolenic acid (GLA), evening primrose oil is being used in increasing amounts in nutritional and pharmaceutical preparations, and may alleviate various chronic disease states.[19-21] Therefore, effects of intervention may appear to possess immune-modulatory and anti-inflammatory roles; and inhibit increase in pro-inflammatory cytokines and may represent novel therapeutic strategies against MS. This study is designed to evaluate correlations between expanded disability status scale and cytokines after intervention with co-supplemented hemp seed and evening primrose oils and hot-natured diet in patients with relapsing remitting MS.

Materials and methods

This trial was carried out on 23 relapsing remitting MS patients to evaluate correlations between expanded disability status scale and cytokines after intervention 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,[22] with a type of relapsing remitting MS, and ages 14-55 years were enrolled. Patients with secondary or primary progressive MS, pregnancy, corticosteroid treatment, patients suffering 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 questionnaire (nqFFQ) to assess food and drinks' consumption and dietary habits. They were asked to maintain their usual level of physical activity and not to consume any supplements during the study. We must notice that the co-supplemented oils (combination of hemp seed and evening primrose oils with 9:1 ratio) are foodstuffs and without side effects. The patients received the co-supplemented oils, 18-21g/day (6-7g, three times daily) with advising hot-natured diet, for 6 months. To achieve this objective, patients were asked to consume hot-natured diet with a wide choice of foods and drink items permitted during each dietary period and delivered at home for 6 months. Hot-natured diet includes foods with hot-natured, 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-natured, 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-natured, avoiding alcohol and smoking. The patients were contacted monthly by telephone to assess compliance. All measures were repeated similarly with same approach and assessors at the end of intervention period.

Measuring disability status of patients

A medical history to check clinical status EDSS was used. The EDSS quantifies disability in eight functional systems (FS) and allows neurologists to assign a functional system score (FSS) in each of them. Scales for the total Kurtzke EDSS[22] are from 0 to 10, in which the 0 score indicates no disability at all and 10 indicates death due to MS.

Blood sample processing and analysis

Plasma and supernatants were tested for cytokines using a sandwich enzyme-linked immunosorbent assay (ELISA). All subjects also provided a 10 ml venous blood sample, collected from the patients before and 6 months after treatment in an EDTA-fortified blood collection tube using a 21-gauge needle. The blood was immediately mixed with the anticoagulant and placed on ice. The sample was centrifuged at 3,000 rpm for 10 min at room temperature, and serum was separated and aliquots were stored up to one year (-80°C) before analysis. The cytokine assay for IL-4, IFN-γ and IL-17 was performed using the ELISA with commercially available kits (U-

CyTech, Netherlands). The absorbance of each well was read at 450 nm. Plasma cytokine concentrations were calculated with standard curve generated from recombinant cytokines.

Statistical analysis

The statistical analysis was performed using SPSS 14 (SPSS Inc, Chicago, IL). Data was expressed as mean ± standard deviation (SD). Pre- and post-intervention comparison in continuous variable was done using paired t-test and Wilcoxon ranked test. Association between continuous variables was assessed using Pearson correlation coefficient. Statistical significance was defined as $p< 0.05$.

Results

Clinical and immunological results

Twenty three relapsing remitting MS patients were enrolled in this study. This study was performed between October 2010 and October 2011. Of the 23 patients, 69.5% (n = 16) were females and 30.4% (n = 7) were males. Participants' characteristics have been presented shown in Table 1.

Table 1. Clinical and demographic characteristics of the study

Age	Average age at onset	Disease duration	Interferon intake	Gender (M/F)
31.2±7.5	25.0±7.5	6.26±3.9	22 (95.7)	7/16

The clinical results of the trial are summarized in (Table 2). There was significantly improvement in EDSS at the end of the intervention. Our intervention reduced the production of pro-inflammatory cytokines (L-17 and IFN-γ, while significantly increased anti-inflammatory cytokine (IL-4) concentration were observed. This data revealed that intervention imposes its beneficial role by decreasing Th1 and promoting Th2 responses in relapsing remitting MS patients after 6 months.

Table 2. Effect of intervention on clinical variables

Variables	EDSS	IL-4	IFN- γ	IL-17
Baseline	2.76±1.39	0.58±.50	0.26±0.04	0.48±0.39
Six months	1.77±1.70	0.69±.69	0.24±0.04	0.41±0.34
p value	**0.001**	**0.027**	**0.001**	**0.009**

The evidence presented the presence of association among EDSS and inflammatory cytokine IL-17 and IFN-γ concentration; moreover, a significant inverse correlation was observed between EDSS and IL-4 in relapsing remitting MS patients (Table 3).

Table 3. The correlation between mean EDSS results and cytokines profile

Cytokines/ EDSS	IL-4	IFN-γ	IL-17
r (p)	-0.504	.217	.459
p value	**.014**	**.019**	**.028**

Discussion

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).[23-25] Hippocrates says "let's our diet be our medicine", and Avicenna said that for each person there are 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.[10,25] The Th cells play a critical role in immune response, in part, through the production of cytokines that provide secondary signals to other cells in the immune cascade. Two major types of Th cell responses have been described. Th1 cells produce IL-2, TNF-α and interferon (IFN-γ) while Th2 cells produce IL-4, IL-5, IL-10, and IL-13.[5,6] These subsets of T-cells interrelate each other's development, with Th1 cytokines suppressing Th2. A subset of T cells that predominantly produces IL-17 has been described.[26] Studies in MS brains found increased transcripts of genes encoding for IL-6, IL-17, and IFN-γ,[27] indicating a potential role for both Th1 and Th17 cells in pro-inflammatory responses in MS. In a similar study, IFN-γ was found to blunt increased production during relapse. [28] Shahabi *et al*, showed the persons of a hot-natured had more deviation of the immune system towards Th2 responses than the persons of a cold-natured, and in agreement with TIM practitioners' view that MS (Th1-mediated autoimmune disease), is more prevalent in cold-natured persons than in hot-natured. Based on studies, an allergen can induce allergic reaction in hot-natured persons with a higher strength than that in cold-natured persons, because the former have a greater tendency to Th2-like responses.[29] Immune responses during infancy and early childhood are dominated by Th2 cytokines, but the shifting toward Th2 pattern decreases with age,[30,31] and an allergen induce allergic reaction in child with a higher strength than that in adults. This is in agreement with TIM's belief that the nature is dominated by warmth at birth but its warmth decreases with age.[10] This critical point that we should indicate why MS attacks is observed in the start of adulthood age. Our study provides both clinical and immunological evidence of decreased MS disease activity during periods of intervention in patients. The evidence presented the presence of association among EDSS and IL-17 and IFN-γ concentration, and a significant inverse correlation between EDSS and IL-4 in patients. IL-4 is produced by activated macrophages and some lymphocytes.[32] It has

many biological roles, including the stimulation of activated B-cell and T-cell proliferation, and the differentiation of CD4+ T-cells into Th2 cells. It is a key regulator in humoral and adaptive immunity. IL-4 induces B-cell class to switch to IgE and overproduction of IL-4 is associated with allergies.[33] In this study, immunological assay confirmed the results of clinical examinations, and depending on Tables 2 and 3, it indicated that patients have a higher rate of deviation of the immune system towards Th2 responses (allergic responses) and were healthier in comparison with baseline, while a hallmark in the pathogenesis of MS is a shift in the ratio of Th cells towards Th1 cells.[34,35] The current studies suggest Th17 immunity plays an important role in MS and blocking this cytokine protects against disease.[7-9] In this regards, anti-inflammatory effect was seen in patients. The results showed a strong trend towards a decrease in pro-inflammatory cytokines IL-17 and IFN-γ and increases in IL-4 was seen in patients. Also, the findings indicated that intervention in patients with hot-natured substances blocks the expression of IL-17 cytokine (Table 2). These results are in agreement with the complications relating to hot or cold natures 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 immune-modulatory and anti-inflammatory roles as the main mechanism of action and Beta-interferons and Glatiramer acetate are on top of this list.[36-38] This would mean less deviation towards Th1 immune responses and may lead to a reduction in disease severity in the patients (Tables 2, 3). Based on TIM practitioners' view, hot-natured food is useful for MS patients, while cold-natured food aggravates their disease. Women are dominated twice more than men by cold nature; that confirms autoimmune diseases such as MS is mostly common in women rather than in men. Also, parameters like weather coldness, lack of sun light exposure and stressful life enhance coldness in subjects.[39] A trend favoring in patients was maintained on EDSS 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, because of limited efficacy and adverse side effects of existing agents.[40,41]

Conclusion

In conclusion, our study demonstrates for the first time in the literature a decrease in both clinical and pro-inflammatory disease activity in MS patients during periods of dietary intervention. Our data demonstrated that co-supplemented hemp seed and evening primrose oils with Hot-natured diet intervention may decrease the risk of developing MS related to the effects on decrease in pro-inflammatory and increase in anti-inflammatory cytokines.

Limitations

Monthly assessment is not enough to ensure patients comply with the treatments. Also, we suggest the trial on more patients and a control group to achieve more strength evidence.

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Competing interests

The authors declare that they have no competing interests.

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