Global Journal of Medical research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 1 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Effect of Antioxidant Vitamins on the Oxidant / Antioxidant Status in Patients with Multiple Sclerosis

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Antioxidant Status in Patients with Multiple Sclerosis, Iraq

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Objective: To evaluate the effect of 3 months supplementation of antioxidants vitamins (ascorbic acid (vitamin C) and alphatocopherol (vitamin E)) on the oxidant / antioxidant status and on the clinical course in patients with relapsing remitting MS (RRMS) and to compare with the placebo therapy and healthy subjects as a control.

Patients & Methods: This is a non randomized single blinded clinical trial was conducted on a total number of 60 patients (24 males and 36 females) with age ranged between 15-54 years, diagnosed to have relapsing-remitting multiple sclerosis (RRMS) and were registered at Neurology Outpatients Department in Ibn Sina Teaching Hospital in Mosul City / Iraq and receiving subcutaneous **β**- interferon, in the period from 1st of February 2012 to the 1st of July 2012.

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GJMR-B Classification : *NLMC Code: QV 704*

Strictly as per the compliance and regulations of:

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Results: There were significantly higher level but a significant lower level of mean serum MDA and TAS concentration respectively in the two MS patients groups (antioxidant vitamins group and placebo group) before starting therapy as compared with the control group. After 3 months antioxidant vitamins therapy, there were a highly significant reduction in the mean serum level of MDA and EDSS but a highly significant increase of the mean serum level of TAS in comparison to their value before therapy. While after 3 months placebo therapy there were insignificant changes in the of mean serum level of MDA and TAS and insignificant difference in EDSS in comparison to their value before therapy. The use of antioxidant vitamins for 3 months resulted in a significant lower level of mean serum MDA, but a significant higher level of mean serum TAS and a significant lower value of EDSS when compared to 3 months use of placebo therapy.

Conclusion: Three months antioxidant vitamins (vitamin C and E) supplementation in RRMS patients causes a significant improvement of the oxidant / antioxidant status and the clinical course of MS patients represented by EDSS. Vitamins (C and E) might be used as adjunct therapy in MS.

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I. Introduction

Multiple sclerosis (MS) is an inflammatory, autoimmune, demyelinating disease of the central nervous system (CNS)[1]. It is characterized by loss of myelin , the fatty tissue that surrounds and protects nerve fibers allowing them to conduct electrical impulses[2]. Although the reasons for the autoimmune demyelination are far to be clear[3], one of these common features is the neuronal imbalance in oxidants/antioxidants[4] .

Central nervous system (CNS) is particularly susceptible to reactive oxygen species (ROS) induced damage due to the high oxygen demands of the brain and low concentration of endogenous antioxidants[5].

Although different mechanisms may contribute to demyelination and neurodegeneration in MS, mitochondrial injury and subsequent energy failure is a major factor driving tissue injury[6-10]. Growing evidence indicates that oxidative stress plays a major role in the pathogenesis of MS[11,12]. Mitochondrial proteins and DNA are highly vulnerable to oxidative damage[13], and it is thus expected that free radical-mediated mechanisms may drive mitochondrial injury in MS [14-16].

Vitamin E acts as a fat-soluble antioxidant against ROS which has been linked to the pathogenesis of MS[17]. In addition, vitamin E has a direct modulatory effect on immune cells. It has been demonstrated to enhance T-cell function in mice by increasing division and interleukin-2 (IL-2) production, as well as reducing prostaglandin E 2 from macrophages[18]. Also vitamin E, a potent antioxidant agent, exerts a protective role as

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free radical scavenger through a non-enzymatic mechanism out of the cell and is the most effective antioxidant agent in lipid structure [19].

Vitamin C prevents the development of experimental autoimmune encephalomyelitis (EAE), an animal model of MS[20] and ameliorates its symptoms[21]. It is likely that vitamins C and E act in a synergistic manner, vitamin E primarily being oxidized to the tocopheroxyl radical and then reduced back to tocopherol by vitamin C[22].

So the aim of this non randomized clinical trial was to assess the effect of supplementation of antioxidant vitamins (vitamin C and E) for three months for patients with RRMS who were treated by β-interferon on the oxidant / antioxidant status and the clinical course of the disease before and after therapy using EDSS.

II. Patients & Methods

Sixty patients known to have RRMS (according to Mc Donald criteria) and registered in the Neurology outpatient Department in Ibn Sina Teaching Hospital and receiving subcutaneous β -interferon 250 μg thrice weekly, enrolled in this study, excluding pregnant and lactating women, people receiving trace elements or vitamin B-complex within one month before the study, smokers and alcohol drinkers or patients with acute or chronic illness rather than MS. These patients were divided into two groups: First group included 30 patients started to receive the antioxidant therapy with vitamin C (ascorbic acid), Cetavit manufactured by Al-shahba Lab. Syria, 500 mg twice daily, and vitamin E (α-tocopherol), Vita E, manufactured by Asia pharmaceutical industries, Syria, 400 I.U. once daily for three months (antioxidant vitamins group). The second group included the other 30 patients, were kept on a placebo therapy in the form of capsules filled with glucose powder twice daily, for 3 months duration (placebo group). Thirty apparently healthy individuals, matched with the patients groups by age and sex and BMI were considered as a control group.

About 5 Blood samples were collected from the patients of the antioxidant vitamins group and the placebo group at the first visit before taking antioxidant vitamins or placebo therapy respectively then after three months of use and from the control group once at the first visit only. The separated serum was kept frozen at – 20°C for measurement of TAS using commercial kit supplied by Randox and MDA using manually prepared reagent and followed Buege and Aust, method[23]. The effectiveness of antioxidants and placebo therapies was assessed in both patients groups by using expanded disability status score (EDSS) [24]. BMI was measured by dividing weight in (kg) by square of height in (meter).

Statistical Analysis: Computer feeding was conducted by prepared computer program SPSS version 18. Standard statistical methods were used to determine the mean and standard deviation (SD). Paired student t-test was used to compare the results for measured biochemical parameters between patients groups and control group. All values quoted as the mean ± SD and P-value ≤ 0.05 was considered to be statistically significant.

The approval of the study protocol by an ethic committee has been obtained from the local health committee of Ministry of Health and College of Medicine - University of Mosul – Iraq.

III. Results

The study sample consisted of 90 individuals with age ranged between 15-54 years. Sixty of them were patients with RRMS and they were equally assigned to two groups, namely antioxidant vitamins group and the placebo group. The other 30 individuals were apparently healthy subjects with age ranged between 20-42 years , were served as control group.

Table (1) shows that there were no significant differences between the characteristic of the two patients groups and the control group, enrolled in this study.

Table 1 : General characteristic of the MS patients and the control

Parameters	Mean ± SD			
	Antioxidant vitamins group n=30	Placebo group n=30	Control group n=30	P-Value
Age(year)	34.93±8.51	35.60±8.88	32.50±5.49	NS
Sex male Female	24 36	12 18	12 18	NS
BMI (Kg/m^2)	26.14±5.95	25.06±4.04	24.16±2.66	NS
Duration of disease (years)	3.90±2.55	3.20±3.91		NS

Duration of β- interferon therapy (years)	2.40±1.49	2.46±2.67		NS
EDSS	3.25±1.54	3.21±1.42		NS

Table (2) demonstrates that the mean serum level of MDA was significantly higher in the two MS patients groups before starting therapy (antioxidant vitamins group and placebo group), whereas the serum level of TAS was significantly lower in these patients as compared with the control group.

Table 2 : Comparison between mean serum level of MDA and TAS of the MS patients groups before starting therapy with the control group

Parameters	Mean ± SD		
	Antioxidant vitamins group before (n=30)	Placebo group before (n=30)	Control group (n=30)
MDA	2.01 ± 0.53 a	1.85 ± 0.51 a	0.90 ± 0.19 b
TAS	0.91 ± 0.40 a	0.93 ± 0.26 a	1.99 ± 0.25 b

- (a , b) different letters (transversely), means significant difference

By the comparison of the mean serum level of MDA, TAS, and EDSS before and after receiving antioxidant vitamins, there were a highly significant decrease in mean serum MDA and EDSS but a highly significant increase of the mean serum TAS of the patients after 3 months antioxidant vitamins therapy, as shown in table (3).

Table 3 : Comparison between mean serum level of MDA, TAS, and EDSS of the antioxidant vitamins group before and after receiving antioxidant vitamins

Parameters	**Mean ± SD**		**P-Value**
	Antioxidant vitamins group before (n=30)	**Antioxidant vitamins group after (n=30)**	
MDA (µmol/l.)	2.00 ± 0.53	1.05 ± 0.56	<0.001
TAS (mmol/l.)	0.91 ± 0.40	1.81 ± 0.41	<0.001
EDSS	3.25 ± 1.54	2.26 ± 1.45	<0.001

Table (4) illustrates that there were no significant differences in the mean serum level of MDA and TAS and EDSS value after receiving placebo therapy for 3 months, when compared with their level before therapy.

Table 4 : Comparison between mean serum level of MDA, TAS, and EDSS of the placebo group before and after receiving placebo

Parameters	**Mean ± SD**		**P-Value**
	Placebo group before (n=30)	**Placebo group after (n=30)**	
MDA (µmol/l.)	1.85 ± 0.51	1.80 ± 0.49	NS
TAS (mmol/l.)	0.93 ± 0.26	1.01 ± 0.38	NS
EDSS	3.21 ± 1.42	3.18 ± 1.33	NS

Table (5) demonstrates that the use of antioxidant vitamins for 3 months resulted in a significant lower level of mean serum MDA, but a significant higher level of mean serum TAS level and a significant lower value of EDSS when compared with 3 months use of placebo.

Table 5 : Comparison of mean serum level of MDA, TAS and EDSS between the antioxidant vitamins group and placebo group after 3months therapy

Parameters	Mean ± SD		P-Value
	antioxidant vitamins group after (n=30)	Placebo group after (n=30)	
MDA (µmol/l.)	1.05 ± 0.56	1.80 ± 0.49	<0.0001
TAS (mmol/l.)	1.81 ± 0.41	1.01 ± 0.38	<0.0001
EDSS	2.26 ± 1.45	3.18 ± 1.33	0.03

IV. Discussion

In this study serum MDA level was found significantly higher in patients with MS of both groups (antioxidant and placebo) before starting therapy, than the healthy control subjects. Several studies have demonstrated an increase in the levels of lipid peroxidation, evaluated by measurement of MDA in plasma, serum and in the cerebro spinal fluid (CSF) of MS patients with respect to healthy subjects [25-28].

Lipid peroxidation has been implicated in the pathogenesis of MS [28]. Increased MDA level which is the consequence of lipid peroxidation and a marker of oxidative stress is an evidence of exaggerated oxidative stress in these patients. Progression of the demyelination process and increase of the MS severity enhanced the intensity of LPO in patients with MS, manifested by increased levels of primary LPO products [2].

Karg *et al.*, [29] and Vinychuk *et al.,* [30] reported that the plasma lipid peroxides levels were increased followed by decreased vitamin E level in MS patients. Koch *et al.,* [31] measured blood plasma lipid peroxidation in different types of MS , they found significantly higher levels in all types compared with the control. Besler and Comoglu [32] found an increase in plasma levels of oxidized lipoproteins and a decrease of antioxidant vitamins in their study on 24 MS patients, and Ferretti *et al.,* [33] reported an increase in plasma lipid peroxidation in patients in an early stage of the disease .

Regarding the measurement of TAS, which is better than measurement of individual antioxidant enzyme , because it reflects the whole antioxidant status of the body, and still another antioxidants substances not discovered yet. Therefore measurement of plasma total antioxidant capacity may give a more precise indication of the relationship between antioxidants and disease[2]. In this study TAS concentration was lower in MS patients, when compared with the control group. The results in this study are in accordance with the results of some previous studies that compared the concentrations of TAS and individual antioxidant enzymes in MS patients [2, 28, 34,35]. Choi *et al.,* [36] reported that glutathione (GSH) levels were lower in patients with MS, while Miller *et al.,* [37] found a low levels of superoxide dismutase (SOD) in patients with MS .

Jimenez-Jimenez *et al.,*[38] compared the serum levels of vitamin E in 36 patients with MS and 32 matched controls. They found that the serum level of vitamin E was significantly lower in patients with MS than controls. Besler *et al.,* [27] found that vitamin E levels were significantly lower in 24 patients with MS than in 24 controls. Salemi *et al.,* [39], found significantly lower levels of vitamin E in 40 patients with MS than in 80 healthy controls. The decrease of the concentration of vitamin E, the major hydrophobic chain-breaking antioxidant, confirms the possible involvement of this vitamin in MS pathology.

Vitamin C (ascorbic acid), has antioxidant properties. Its level is decreased in the blood of patients with MS during a relapse compared with those in the remitting phase, which might point to increased antioxidant demand during active demyelination [27].

Central nervous system (CNS) is particularly susceptible to ROS induced damage due to the high oxygen demands of the brain and low concentration of antioxidants, both enzymatic and non-enzymatic antioxidants . It is suggested that antioxidant status was altered and low activity of antioxidant enzymes were observed in CNS of MS patients . Enzymatic and non enzymatic antioxidants could regulate function of different immunologic cells in MS. In addition, an impairment of antioxidant defense systems in MS patients may results in their higher susceptibility to ROS and cause damage of CNS [28] .

The question arises whether oxidative stress in MS, contributes to pathology or whether it is a non-specific epiphenomenon. Evidence for an important role of oxidative stress in the pathogenesis of this disease

should come from clinical trials with antioxidant drugs. However, clinical trials of antioxidant treatments in MS and other neurodegenerative diseases are lacking[34,40].

Data obtained from the present study demonstrated a beneficial effects of the administration of vitamins C and E combination on MDA and TAS levels (significant lowering of MDA, and significant raise of TAS) in MS patients, and improvement in the mean EDSS from 3.25±1.54 to 2.26 ±1.45 with mean difference -0.98±0.77, compared with the placebo group which show no such improvement (a mean difference of -0.13±0.31).

Review of literature provides limited information on the usefulness of vitamin E and vitamin C in patients with MS. The effects of antioxidant vitamins on the course of MS have not been formally assessed in humans, but animal studies provide some rationale for a role in MS[41]. Therefore according to the author knowledge, this study is the first that concerned with the use of vitamin C and vitamin E in patients with MS, because all available studies were done in animal models.

Several animal studies have been performed on the effect of vitamin E on de- and remyelination. The first study[42] used the ethidium bromide demyelinating model. The authors found that treatment with vitamin E and ebselen (an organo-selenium compound possessing antioxidant property) protected against demyelination caused by ethidium bromide. Furthermore, they described that ebselen and vitamin E interfered with the cholinergic neurotransmission by altering acetylcholinesterase activity in the different brain regions and in the erythrocytes. In a follow-up study in the ethidium bromide demyelinating model, it was demonstrated that vitamin E reduced the ethidium bromide-induced damage and increased the endogenous remyelination of hippocampus in rats [(43)]. Also vitamin E attenuates demyelination and potentiates remyelination in animal models of toxin mediated demyelination [42-43].

In conclusion: Three months antioxidant vitamins (vitamin C and E) supplementation in RRMS patients causes a significant improvement of the oxidant / antioxidant status in patients with RRMS patients and a significant improvement of the clinical course of MS patients represented by EDSS.

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