

Influence of Vitamin E Supplementation on Aging

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Abstract

Background: Despite massive and costly efforts, progressive deterioration of biomolecules due to augmented oxidative stress with advancing age has yet not been controlled. It is conceivable that exogenous vitamin E supplementation ameliorate the modifiable indexes via regulating free radical production and consumption of antioxidant reserve.

Aim: The objectives of present study were to investigate the therapeutic effect of vitamin E supplementation in ameliorating the altered activity of antioxidant enzymes (superoxide dismutase, ceruloplasmin & glutathione peroxidase) and erythrocyte malonaldehyde level (MDA, i.e. marker of lipid peroxidation) in middle aged and elderly people.

Material & Methods: Antioxidant enzymes status & MDA levels were estimated by using standard methods in 30 healthy younger individuals (20-30 years) who served as controls and in 60 healthy subjects categorized into two groups i.e. group I (40-55 years) and group II (≥ 56 years) before and after 3 months of vitamin E supplementation. The obtained values were compared statistically by using student's t-test.

Result: Marked reduction in erythrocyte superoxide dismutase activity and glutathione peroxidase were observed in group I ($p < 0.05$) and group II ($p < 0.05$), after vitamin E supplementation. On the other hand, significant increased levels of erythrocyte MDA were observed in group I and group II ($p < 0.001$) which ameliorate significantly ($p < 0.05$) after vitamin E supplementation. However, plasma ceruloplasmin levels were increased significantly only in group II ($p < 0.05$) but remain unaltered ($p < 0.1$) on vitamin E supplementation.

Conclusion: Exogenous vitamin E supplementation provides protection against oxidative stress mediated aging processes and diet rich in vitamin E should be increased with advancing age.

Keywords: Aging, superoxide dismutase, ceruloplasmin, malonaldehyde, glutathione peroxidase.

Introduction

Oxidative damage induced by reactive oxygen species is caused by increased production of superoxide anion (O_2^-) and its metabolites or by reduced bioavailability of antioxidant defenses. The imbalance between pro-oxidants and antioxidants gives rise to cellular oxidative stress, which plays an important role in the progression of aging processes.¹ Reactive oxygen species may act through several mechanisms to mediate major interrelated derangements of cell

metabolism such as peroxidation of lipids, DNA strand breakage, rise in intracellular free Ca^{2+} , damage to membrane ion transporters and other specific proteins.²

Lipid peroxidation is a free radical process in which the polyunsaturated fatty acids contained in LDL or present in cell membrane are degraded to a great variety of aldehydes (i.e. malonaldehyde, propanal and hexanal etc). As a result, excess binding of aldehyde to cellular protein may alter cellular function, membrane permeability, electrolyte balance and thereby leading to progressive deterioration of biological system associated with aging process.³

Protection of cells from potentially injurious superoxide anion (O_2^-) is provided by superoxide

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dismutases, intracellular (CuZnSOD and MnSOD) and extracellular enzymes (E.C. SOD) that catalyse the dismutation of O_2^- to H_2O_2 and O_2 .³ In addition, the action of cytoplasmic SOD in scavenging O_2^- and in inhibiting O_2^- mediated reactions can be mimicked by other copper-containing plasma protein i.e. ceruloplasmin. The antioxidant property of ceruloplasmin is due to its ferroxidase activity towards ferrous ions and, it can prevent the generation of OH^\cdot radicals via Haber's reaction.⁴ Hydrogen peroxide (H_2O_2) formed from superoxide anion, either in presence of transition metal (Fe^{2+} & Cu^{2+}) produces highly toxic hydroxyl radical or produces hypochlorous acid (HOCl) by the action of enzyme myeloperoxidase in neutrophils and macrophages which amplify further destruction. Glutathione peroxidase (GSHPx), a selenium containing enzyme, catalyses the decomposition of H_2O_2 with the help of reduced glutathione and prevents the oxidation of lipids and phospholipids.⁵ Alterations in the levels of these enzymes and increased levels of lipid peroxides further amplify the disturbances in biological processes with increasing age.

A wide range of antioxidants both natural and synthetic have been proposed for treatment of age related biochemical and physiological alterations related to oxidative stress. Increased oxidative stress is the result of either an increased production of free radicals or a depletion of endogenous antioxidants. Considerable attention has been devoted to the potential use of α -tocopherol, a potent chain breaking antioxidant, in the prevention of age related alterations.⁶ Although in previous epidemiological and experimental animal studies, vitamin E reduces oxidative stress mediated damages and facilitates various physiological activities, the mechanism underlying its effect in ameliorating the enzyme activities and levels of MDA (i.e. marker of lipid peroxidation) in different age group subjects have not been fully elucidated.^{7,8} Therefore, the objective of present study was to investigate the therapeutic effect of vitamin E supplementation in replenishing the antioxidant enzyme activity and in controlling the progression of lipid peroxidation in different age group subjects.

Material and Methods

In the present study, 90 healthy subjects of either sex (15 males & 15 females in each group) and different age groups were included from urban area of Allahabad city after taking their informed consent and approval

of protocol by ethics committee of college. These subjects were selected randomly and categorized into three groups depending upon age i.e. control group (younger people) included 30 healthy subjects of age group 20–30 years, group I included 30 healthy subjects (middle aged people) of age group 40-55 years and group II included 30 healthy subjects (elderly people) of age group 56 years onwards. A general information or pre-experimental questionnaire regarding demographic information, family history and limited physical examination was completed from all subjects. Patients with diabetes mellitus, hypertension, renal insufficiency, hepatic disease or under any medicinal treatment were excluded. Height and weight were measured with subject barefoot and light dressed. The body mass index (BMI) was calculated as $BMI = \text{weight (kg)} / \text{height (meter)}^2$. Obese ($BMI > 25$), hypertensives ($BP > 120/80$ mmHg) and smokers were excluded from the study.

Fasting blood samples were collected in EDTA vials from the anticubital vein of the subjects and processed immediately. Antioxidant enzymes and MDA levels were estimated before (non supplemented group i.e. Group I A & Group II A) and after 3 months of vitamin E supplementation (200 mg/day; Group I B & Group II B) and compared it with that of younger controls. Erythrocyte SOD activity was measured by Marklund and Marklund's method.⁹ This method is based on the presence of superoxide anions. The enzyme SOD inhibits the autooxidation of pyrogallol by catalyzing the breakdown of superoxide. The inhibition of pyrogallol oxidation by SOD is monitored at 420 nm and the amount of enzyme producing 50% inhibition is defined as one unit of enzyme activity.

Plasma ceruloplasmin level was estimated by Ravins's method.¹⁰ Ceruloplasmin due to its oxidase activity, catalyses the oxidation of substrate p-phenylenediamine chloride into purple coloured oxidation product which is measured spectrophotometrically.

GSHPx activity was estimated by Beutler's method, after preparation of hemolysate.¹¹ GSHPx catalyses the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) by H_2O_2 . The rate of formation of GSSG is measured by means of glutathione reductase reaction in which NADPH is oxidized and is measured at 340 nm.

Erythrocyte malonaldehyde (MDA) level was

measured as thiobarbituric acid reactive substances, after preparation of hemolysate.¹² The heat induced reaction of malionaldehyde (MDA) with thiobarbituric acid (TBA) in the acid solution forms a trimethine coloured substance, which is measured spectrophotometrically at 532nm. Values were expressed as Mean \pm SD. The significance of mean difference between groups was compared by using Student's t test and distribution of probability (p).

Result

The mean blood pressure and demographic indices of the study groups are depicted in Table 1. The observations made reveal significant changes in antioxidant enzyme status and MDA levels before and after vitamin E supplementation as represented in Table 2 and 3. Erythrocyte SOD activity was significantly low in Group I A (29.5 %; p<0.05) and Group II A subjects (33.8 %; p<0.05) as compared to younger controls. It was found to be increased significantly in Group I B and Group II B (p<0.05; 23.0 % & 21.8 % respectively) on vitamin E supplementation. Plasma ceruloplasmin levels were significantly high only in

Group II A subjects (20.8 %; p<0.05) whereas insignificant change occur (p<0.1; 10.3 %) in Group I A subjects. However, these levels remain unchanged in study group subjects (p<0.1) after vitamin E supplementation. Erythrocyte GSHPx activity was significantly low (22.5%; p<0.05) in Group I A and (29.92 %; p<0.05) in Group II A subjects as compared to healthy controls. It increased significantly (p<0.05; 21.6 % & 19.4 % high respectively) in both groups after vitamin E supplementing. Similarly, significantly elevated levels of erythrocyte malionaldehyde (MDA) were observed in non supplemented groups (i.e. 23.8 % high in Group I A & 37.3 % high in Group II A; p<0.001) compared to healthy controls. In supplemented group, MDA levels decreased significantly (p<0.05; 27.7 % & 23.5 % low) in Group I B and II B, as compared to non-supplemented group.

Discussion

Excessive ROS generation leading to oxidative stress and damage of cellular biomolecules (proteins, lipids, and nucleic acids) has been hypothesized to be the major contributor to aging process and many

Table 1: Demographic profile of study subjects (Mean \pm SD)

S No	Particulars	Control group (n=30)	Group I (n=30)	Group II (n=30)
1)	Age (years)	23.5 \pm 5.0	45.0 \pm 7.0	67.0 \pm 9.0
2)	M:F ratio	1:1	1:1	1:1
3)	Height (meter)	1.58 \pm 0.07	1.54 \pm 0.06	1.60 \pm 0.08
4)	Weight (kg)	55.2 \pm 3.8	57.0 \pm 2.9	59.4 \pm 3.4
5)	BMI (kg/m ²)	22.0	24.3	23.6
6)	Systolic blood pressure (mmHg)	104 \pm 6.0	110 \pm 5.4	120 \pm 4.8
7)	Diastolic blood pressure (mmHg)	72.0 \pm 3.0	76.0 \pm 4.2	70.0 \pm 3.4

Table 2: Antioxidant enzymes and Malionaldehyde levels in different age group before vitamin E supplementation. (Mean \pm SD)

S No	Particulars	Control group(n=30)	Group I A(n=30)	Group II A(n=30)
1.	SOD level(U/gm Hb)	1970.24 \pm 251.7	1389.02 \pm 197.42 **	1304.3 \pm 172.36 **
2.	Ceruloplasmin(mg%)	22.9 \pm 7.3	25.28 \pm 6.3 *	27.5 \pm 8.0 **
3.	GSHPx(IU/gm Hb)	38.4 \pm 8.3	29.76 \pm 6.0 **	26.92 \pm 5.6 **
4.	Malionaldehyde (μ molMDA/ml)	2.78 \pm 0.26	3.44 \pm 0.33 **	3.81 \pm 0.35 ***

* p<0.1 : Non significant ** p<0.05 : Significant *** p<0.001 : Highly significant

Table 3: Antioxidant enzymes and Malionaldehyde levels in different age group after vitamin E supplementation. (Mean \pm SD)

S No	Particulars	Control group(n=30)	Group I B(n=30)	Group II B(n=30)
1.	SOD level(U/gm Hb)	1970.24 \pm 251.7	1708.49 \pm 204.3**	1588.6 \pm 188.0**
2.	Ceruloplasmin(mg%)	22.9 \pm 7.3	24.53 \pm 4.0 *	26.84 \pm 6.2 *
3.	GSHPx(IU/gm Hb)	38.4 \pm 8.3	36.19 \pm 7.2 **	32.16 \pm 6.4 **
4.	Malionaldehyde (μ molMDA/ml)	2.78 \pm 0.26	2.49 \pm 0.20 **	2.92 \pm 0.28 **

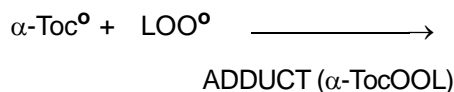
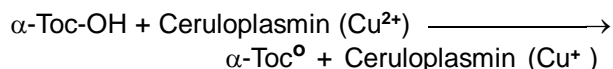
* p<0.1 : Non significant ** p<0.05 : Significant *** p<0.001 : Highly significant

diseases, such as cardiovascular diseases and cancers.^{1, 2} In particular, altered antioxidant enzyme activities and lipid peroxidation are considered to be a major phenomenon by which ROS can cause tissue damage leading to impaired cellular function and alterations in physicochemical properties of cell membranes, which in turn disrupt vital functions.¹³ Several evidences have documented overproduction of free radicals with increasing age.^{1, 14}

Vitamins directly scavenge ROS and regulate the activities of antioxidant enzymes. In this context, vitamin E has been recognized as one of the most potent lipophilic chain breaking antioxidants.³ Although few studies explicitly show the effects of vitamin E on the activities of antioxidant enzymes, the mechanism underlying its effect in ameliorating the enzyme activities and levels of MDA in different age group subjects have yet not been fully elucidated.^{6, 15} In the present study, we observed that superoxide dismutase activity in both middle aged and elderly subjects were significantly low (p<0.05) compared to healthy controls. It was found to increase significantly with vitamin E supplementation (p<0.05). However, Li et al, have found no effect of vitamin E supplementation on SOD activity.¹⁷

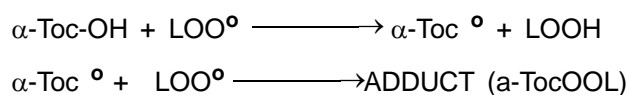
Superoxide anion scavenging action of SOD can be mimicked by other copper containing enzyme ceruloplasmin, which also has the capacity to scavenge O₂⁻. In the present study, plasma ceruloplasmin levels were found to be significantly high only in elderly (p<0.05) whereas insignificant change occur (p<0.1) in Group I A subjects. However, these levels remain unchanged in study group subjects (p<0.1) after vitamin E supplementation. On vitamin E supplementation, its level remain unaltered (p<0.1) in supplemented group, which were in concordance with findings of Reunanen et al.¹⁸ Although, the precise mechanism involved

behind this result is still unknown, it could be explained on the basis of pro-oxidant property of vitamin E with ceruloplasmin by which it converts Cu²⁺ of ceruloplasmin to Cu⁺ which in turn catalyses lipid peroxide (LOO^o) production from lipid hydroperoxide (LOOH) and itself reduced back to Cu²⁺. On the other hand, α tocopherol radical and LOO^o radical, formed during this step is utilized in the formation of adduct as reported by Maiorino et al.¹⁹



Moreover, GSHPx plays a crucial role in the final detoxification of H₂O₂. It spontaneously reacts with and scavenges many forms of ROS, prevents oxidation of lipids and phospholipids, maintains intracellular redox milieu, replenishes a number of crucial antioxidants (vitamin E and C) and produces vasodilatory prostacyclin by the endothelium to maintain normal blood pressure.¹⁵ In the present study, low GSHPx activities were observed in both middle age and elderly subjects as compared to younger controls which on vitamin E supplementation, increased significantly in both the supplemented groups. It could be explained as a glutathione sparing action of vitamin E by inhibiting lipid peroxidation and thereby replenishes GSHPx activity.²⁰ Recently, similar findings were reported by Jaiswal et al and Garg et al, in their vitamin E supplementation studies on subjects of different age related complications.^{15,21} Conversely, Li et al had found no effect of vitamin E supplementation on GSHPx activity.¹⁷

Above mentioned observation is well supported by marked reduction in MDA levels ($p < 0.05$) in supplemented group subjects which was 23.8% & 37.3% high in non-supplemented middle age and elderly subjects respectively. These findings clarify the chain breaking antioxidant property of vitamin E by which it protects the membrane bound lipids and nascent LDL against free radical mediated lipid peroxidation (i.e. key factor of atherosclerosis), prevents lipid peroxidation mediated destruction in cell, subcellular organelles and biomolecules and thereby plays a significant role in the reduction of age associated destructive events.



Furthermore, its cardioprotective, antihypertensive, anti-inflammatory, analgesic activity and its role in extending the life span of non-vertebrates, in preventing neurological dysfunction, memory or slow thinking problems, skin problems and cataract formation in elderly have been well documented.²²

Conclusion

In view of substantial evidence from previous studies and present findings supporting the critical role of vitamin E in amelioration of antioxidant enzyme activity and prevention of lipid peroxidation, the present study is strong enough to convince nutritionist that daily consumption of diet rich in vitamin E should be increased with advancing age. In addition, present study authenticates the fact that oxidative stress plays a crucial role in aging process which can be regulated by exogenous antioxidant supplementation. However, more work is needed to shed light on the therapeutic use of vitamin E.

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