The effect of antioxidant vitamins E and C on cognitive performance of the elderly with mild cognitive impairment in Isfahan, Iran: a double-blind, randomized, placebo-controlled trial

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Abstract

Purpose This study was carried out to investigate the effect of vitamins E and C on cognitive performance among the elderly in Iran.

Methods About 256 elderly with mild cognitive impairment, aged 60–75 years, received 300 mg of vitamin E plus 400 mg of vitamin C or placebo daily just for 1 year.

Background Demographic characteristics, anthropometric variables food consumption, cognitive function by Mini-Mental State Examination (MMSE), and some of the oxidative stress biomarkers were examined.

Results Antioxidant supplementation reduced malondialdehyde level (P < 0.001) and raised total antioxidant capacity (P < 0.001) and glutathione (P < 0.01). The serum 8-hydroxydeoxyguanosine remained unchanged (P < 0.4). After adjusting for the covariates effects, MMSE scores following 6- (25.88 ± 0.17) and 12-month antioxidant supplementation (26.8 ± 0.17) did not differ from control group (25.86 ± 0.18 and 26.59 ± 0.18, respectively).

Conclusion Despite significant improvement in most of the oxidative stress biomarkers, antioxidants’ supplementation was not observed to enhance cognitive performance. A large number of kinetic and/or dynamic factors could be suspected.

Keywords Supplementation · The elderly · Mild cognitive impairment · Vitamin E · Vitamin C

Introduction

Dementia is defined as a progressive loss of brain cognitive function that mainly affects older people. It can be occurred by a number of etiologies, which lead to a decline in memory, judgment, decision making, learning, executive functioning, and other mental activities. The symptoms would cause clinically significant distress or impairment in social, occupational, or other important areas of functioning [1]. Alzheimer’s disease, for example, is the most known cause of dementia, characterized by severe cognitive and functional disabilities [2]. As a slowly progressive process, cognitive decline in its early stages may not impair important area of functioning. Mild cognitive impairment (MCI) is another term, which has been defined as a transitional stage between normal cognitive function and dementia. People with MCI have minor problems with memory, attention, speech, decision making, visuospatial, and psychomotor functions, which exceed those expected
for their age and educational level [3]. However, there is no sign of major impairment in social, occupational, or personal functioning.

The patients suffering from MCI have a significantly higher rate, about tenfold, of progression to Alzheimer’s disease compared to cognitively normal elderly people [4, 5].

Despite considerable investigations, the exact factors associated the induction of MCI or its more rapid progression to Alzheimer’s disease has not been well defined. Extensive evidence indicates that oxidative stress plays an important role in the pathogenesis of cognitive disorders. Oxidative stress has been defined as a state where highly reactive free radicals activity exceeds the antioxidant systems defense. This can lead to chemical oxidative modification of macromolecules and change the structure and function of cellular key components [4, 6, 7].

Increased level of oxidative stress markers including malondialdehyde (MDA) [4, 8] and (8-hydroxydeoxyguanosine) 8-OHdG [9] and decreased levels of glutathione (GSH) [10], total antioxidant capacity (TAC) [11], and antioxidant enzymes [4] have been reported in MCI patients. Cerebral tissue is particularly susceptible to reactive oxygen species (ROS) damage due to its high oxygen requirements for metabolism, low content of antioxidants, and a high content of polyunsaturated fatty acids [4, 6, 7]. These oxidative events have been implicated both in MCI [12] and its progression to Alzheimer’s disease [13].

Regarding these observations, antioxidant vitamins, including vitamins E, the most potent lipophilic chain-breaking antioxidant [14], and vitamin C, a potent watersoluble antioxidant, are expected to reduce neuronal damage and improve cognitive performance of these patients; however, previous studies have yielded conflicting results in this regard. While some clinical and epidemiological studies pointed to the slowing of cognitive decline with the use of vitamins C and/or E [15–19], some others failed to find any protective effect for these antioxidants [20–22].

The aim of the present study was to examine the effects of antioxidant vitamins E and C supplements on cognitive performance in a group of elderly males and females in Iran.

Subjects and methods

Subjects and study design

This study was designed as a double-blind, randomized, controlled clinical trial. From retirees clubs, 761 elderly volunteers with ages between 60 and 75 years were recruited. Exclusion criteria included obvious disabling disease, Alcohol intake, smoking, and routine consumption of neurological or antioxidants drugs. An expert psychologist evaluated the subjects to find those suffering from MCI on the basis of attaining 21–26 scores in Mini-Mental State Examination (MMSE). The Iranian version of MMSE had been validated and found to be applicable for the local elderly population [23]. Of the 761 volunteers, 296 were found to have MCI and selected for the next part of the study. From these, 40 did not continue with the study due to the problems tolerating the supplementations (14 subjects), 1 subject died, and 25 subjects abstained to continue participation due to personal reasons. Therefore, 256 subjects completed the study.

As shown in Fig. 1, finally selected subjects were randomly assigned into two groups, namely intervention and control. Selection and grouping were performed using stratified method followed by simple randomization method.

The present study protocol was conducted in accordance with the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee at Ministry of Health and Medical Education, Iran. To participate in this study, all the subjects or their families signed the consent forms.

Procedures

One group consumed 300 mg of vitamin E (Dl-alpha-tocopherol acetate) plus 400 mg vitamin C (ascorbic acid) per day, and the second group consumed placebo with the identical condition over the 1-year intervention period.

Fasting venous blood from each subject was obtained at three time points, namely on days 0, 180, and 360. Serum MDA was measured according to the method described by Yu et al. [24]. TAC was assessed based on Miller and Rice-Evans [25]. Red blood cells GSH was determined quantitatively using PerkinElmer spectrophotometer according to the method of Beutler et al. [26]. 8-OHdG was measured using a competitive enzyme-linked immunosorbent assay (ELISA) kit from Immundiagnostik Company, Germany. Cognitive performance was evaluated by MMSE at three time points similar to blood sampling. Three-day dietary record forms were also completed for each participant by a trained nutritionist every 2 months throughout the study.

Data analysis

The results are presented as mean ± standard error of mean (SEM) for quantitative variables and number (percent) for qualitative variables. Repeated measures analysis of variance (ANOVA) was used for analyzing the data as the main statistical method. Mauchly’s sphericity test was conducted to assess sphericity as a perquisite assumption.
When the assumption was not satisfied, Huynh–Feldt correction was used. Within-group comparisons at each follow-up time point were made using repeated contrasts. Between-group comparisons were conducted using two independent samples $t$ test. Chi square test for difference between proportions was used. Analysis of the data was done by SPSS software version 19. Analysis of consumed foods was done by Food Processor II software. For all hypotheses, a significance level of $P < 0.05$ was considered statistically significant.

Results

General characteristic of the subjects

General characteristics of the studied subjects for both groups are summarized in Table 1. As can be seen, no significant differences were found between supplemented and control groups (Student’s $t$ test or Chi square tests).

Data from 3-day dietary record for intakes of vitamin C, vitamin E, carotene, selenium, and zinc were analyzed (Table 2) to compare the dietary antioxidant

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**Table 1** Descriptive characteristics of the subjects at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Supplemented (n = 127)</th>
<th>Control (n = 129)</th>
<th>$P$ value*</th>
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<tr>
<td>Age (year)</td>
<td>66.5 ± 0.39</td>
<td>66.3 ± 0.38</td>
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<tr>
<td>Weight (kg)</td>
<td>71.0 ± 0.94</td>
<td>69.1 ± 0.94</td>
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</tr>
<tr>
<td>Height (cm)</td>
<td>162.5 ± 0.78</td>
<td>162 ± 0.86</td>
<td>0.703</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>26.9 ± 0.35</td>
<td>26.3 ± 0.35</td>
<td>0.233</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>141.2 ± 1.67</td>
<td>137.4 ± 1.7</td>
<td>0.119</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>80.2 ± 1.2</td>
<td>77.9 ± 1.2</td>
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<thead>
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<th>Sex</th>
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</thead>
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<tr>
<td>Male n (%)</td>
<td>63 (49.6)</td>
<td>57 (44.2)</td>
<td>0.229</td>
</tr>
<tr>
<td>Female n (%)</td>
<td>64 (50.4)</td>
<td>72 (55.8)</td>
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<table>
<thead>
<tr>
<th>Educational level</th>
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<tbody>
<tr>
<td>Primary n (%)</td>
<td>25 (19.8)</td>
<td>16 (12.5)</td>
<td>0.127</td>
</tr>
<tr>
<td>Secondary n (%)</td>
<td>17 (13.5)</td>
<td>10 (7.8)</td>
<td></td>
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<tr>
<td>Diploma n (%)</td>
<td>48 (38.1)</td>
<td>55 (43.0)</td>
<td></td>
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<tr>
<td>University degree n (%)</td>
<td>36 (28.6)</td>
<td>47 (36.7)</td>
<td></td>
</tr>
</tbody>
</table>

* Student’s $t$ test or chi-square test

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**Fig. 1** Stratified and simple randomization
intake between the two groups. The independent \( t \) test did not show any statistically significant differences, except for vitamin C in second time point assessment \((P = 0.029)\).

**Oxidative stress biomarkers**

**Mda**

Malondialdehyde level raised significantly within each group during the first 6 months, followed by a significant decline in second half-year of the study \((P < 0.001, \text{ repeated measures ANOVA})\). Mean values of MDA at second time point between supplemented \((2.37 \pm 0.055, P < 0.02)\) and control group \((1.75 \pm 0.067, P < 0.001)\) and third time point \((2.54 \pm 0.055 \text{ and } 2.17 \pm 0.067)\) were significantly different.

**Tac**

The TAC significantly increased during this study within both groups \((P < 0.001)\). Regarding between groups, it
reached to a significantly higher level in antioxidant received subjects compared with controls only at twelfth month of supplementation ($P < 0.001$).

**GSH**

In GSH, within supplemented group using repeated contrasts showed significant differences between second and third time points ($P = 0.02$), while in control group, no significant differences were observed at all time points.

The red blood cells glutathione was also significantly higher in supplemented group only at twelfth month of intervention compared with the control group ($P < 0.01$).

**8-OHdG**

The test showed that the difference of within group for 8-OHdG at second and third time point measurements compared with zero time was significant ($P < 0.001$), but it did not show any significant differences between the two groups throughout the study ($P < 0.4$). The results for the above biomarkers have been shown in Table 3.

**Mini-Mental State Examination (MMSE) score**

After adjusting for potential covariates including age, sex, BMI, blood pressure, educational levels, and dietary antioxidants intake, repeated measures ANOVA of the MMSE scores showed a significant ($P < 0.001$) increasing trend within both supplemented and control groups throughout this study, but no significant differences between these groups were found ($P = 0.88$; Fig. 2).

None of the mean values of MMSE score in 6th and 12th month of intervention between supplemented and control groups (In sixth month: supplemented vs. control $25.88 \pm 0.17$ vs. $25.86 \pm 0.18$ and in 12th month $26.8 \pm 0.17$ vs. $26.59 \pm 0.18$) were significant.

**Discussion**

During 1-year, double-blind, randomized, controlled clinical trial, in spite of improvements in MDA, TAC, and GSH levels, no beneficial effect of antioxidants on cognition was observed at MMSE cognitive assessment.

These markers are some of the most widely used tests of oxidative stress status and at least provide some indications of the bioavailability of administrated antioxidants, although changes in serum level of these markers could not be considered as a solid indication of redox status, especially at the exact target points of brain where antioxidants are expected to exert their possible cognitive effects.

These results are in accordance with some parts of the previous studies, but disagree with others. Even detrimental consequences for antioxidants administration in MCI have been reported [56].

The real causes of these discrepancies among various experiments are unknown, but a long list of involving factors could be suspected. Duration of intervention, types and doses of antioxidants used, the efficacy of cognitive assessment tools, and the influence of confounding variables are some of the factors that alone, or in combination with each other, could potentially affect the outcomes. The quality of studies, especially the number of involving subjects, should also be added to the above list.

Morris et al. [27] obtained positive results regarding the effect of vitamins E and C in Alzheimer disease. In their study, confounding factors may have adversely influenced and the results had been ignored. Alzheimer’s disease has been reported to be associated with educational attainment [28, 29], body mass index, and lifestyle factors such as physical activity [30, 31], older age, and sex [29, 32].

In our trial, all confounding variables were assessed and showed not to be statistically different in supplemented and control groups.

Duration of intervention may be a major difference between our and some of the similar studies and may be one of the causes of difference in responsiveness to antioxidants supplementation. Grodstein et al. [18], who obtained the positive results, argued that the prolongation of antioxidant administration would increase the likelihood of appearance of their cognitive beneficial effect. Nevertheless, in some studies, even in spite of relatively long intervention period of 10 years [33], 3 years [22], 4 years [34] 5 years [35], no positive results were found. Therefore, supplementation duration alone may not always be a significant factor in effectiveness of antioxidants and must be noted along with other influencing factors. It is not clear
that the prolongation of antioxidant supplementation beyond that of our study may result in any positive effect on cognition.

In our trial, we selected 1-year period of antioxidant supplementation, and from our view point, it was an average duration considering the previous similar studies.

Dosage of antioxidants can also be another important factor that seems to be remarkably effective in outcomes of this and similar studies.

The relationship between antioxidants doses and cognitive performance in different studies has not been straightforward. These includes the negative result with 400 mg vitamin E alone [33], 1,400 mg vitamin E + 10 mg donepezil [22] or 540 mg/d of vitamin E + 500 mg/d of vitamin C + 900 mg/d of α-lipoic acid + 400 mg of coenzyme Q [36], and those including positive result with 270 mg vitamin E, 500 mg vitamin C [27] or 1,400 mg Vitamin E + 10 mg Selegiline [21]. In regard to the above studies, some trials in spite of being categorized among high-dose users of antioxidants were not able to obtain positive result while some studies have been revealed negative results by using higher doses. Therefore, dosage of antioxidants cannot be the solely determinant of the outcome and must be noticed along with other effective variables.

Higher dose of vitamin E (> or = 270 mg/d) may increase mortality [37, 38] or increase the risk of prostate cancer [39]. High doses of vitamin C supplementation have been reported to be associated with a higher risk of age-related cataract [40] or hyperoxaluric nephropathy and progressive renal failure [41]. In our study, supplemented group consumed 300 mg of vitamin E and 400 mg vitamin C per day. Vitamin E is considered relatively safe compared to other fat-soluble vitamins. Using a combination of vitamins E and C with a medium dose did not produce any unexpected side effects on subjects.

Type of antioxidants used is another important factor that may influence the results. The selection of proper type(s) of antioxidant(s) for a study such as ours has always been a challenge. There is a long and increasing list of substances in aging [55] should also be noted. Ineffective side effects on subjects.

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unresponsiveness. Further studies especially with more efficient cognitive evaluation tools as well as more diverse antioxidant types may be needed to clarify the possible antioxidant–oxidative stress cognition interactions.

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Conflict of interest: The authors declare that there are no conflicts of interest.

References


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