LOWERED PLASMA VITAMIN C, BUT NOT VITAMIN E, CONCENTRATIONS IN DEMENTIA PATIENTS

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Abstract: Background: Alzheimer’s disease (AD), according to the free radical hypothesis, affects brain regions where free–radical damage occurs. Antioxidant nutrients may help to protect these brain regions. Objective: To investigate whether plasma vitamin C and E status is lowered in subjects with AD and dementia. Design: A case control study was conducted in 93 institutionalized subjects aged 65 + yrs. The dementia group (N = 43) included 15 subjects with Alzheimer’s Disease (AD) and 28 subjects with senile dementia, while the control group included 50 subjects with no cognitive impairment. Subjects with uncontrolled hypertension and/or diabetes were excluded from the study. Plasma vitamin C and E was determined using the 2,6- dichlorophenolindophenol and the HPLC methods, respectively. Dietary intake, including dietary supplements, was assessed using a 2-day plate-waste method. Cognitive function was measured using the MMSE and nutritional status assessed using the Mini Nutritional Assessment (MNA) tool. Results: The control group had significantly higher scores for the MNA, MMSE and Activities of Daily Living, compared with the dementia group. Controls had a significantly higher plasma vitamin C concentration than dementia patients (median = 0.84 (IQR = 0.54) mg/dl and 0.56 (0.80) mg/dl, respectively; P<0.05). The dementia group were more likely to have sub-optimal plasma vitamin C levels (< 0.6 mg/dl) than control subjects (OR = 2.99; 95 % CI = 0.95 – 9.79; P<0.05), despite having similar dietary vitamin C intakes. Plasma vitamin C was positively associated with MMSE score (r = 0.21; P<0.05). No difference was found between the groups for either plasma or dietary vitamin E. Conclusion: Plasma vitamin C levels were lower in subjects with dementia compared to controls, which was not explained by their dietary vitamin C intakes. This data supports the free radical theory of oxidative neuronal damage. Further investigations of whether supplementation with this vitamin may prevent or delay the progression of cognitive decline in patients with AD and senile dementia appear warranted.

Key words: Vitamin C, vitamin E, Alzheimer’s disease, senile dementia, nutritional status.

Introduction

Dementia and Alzheimer’s disease have an increasing medical, social and economic impact, since the elderly are the fastest growing segment of the population (1). Dementia is the most common manifestation of cognitive impairment in older adults, and is estimated to be present in 5 –10 % of subjects aged 65 years and older (2).

There are a number of causes of dementia, the most common one being Alzheimer’s disease, which affects 15 million people worldwide (3) and accounts for approximately two-thirds of all dementia cases (4). The prevalence of the disease doubles every 5 years after the age of 60, with 1 % of individuals at age 60 suffering from AD, compared to over 20 % in those at age 80 (4).

Alzheimer’s disease, according to the free radical hypothesis, is an acceleration of the normal aging process in affected brain regions where free–radical damage occurs (5 - 7). There is evidence to suggest that amyloid- β protein, one of the main protein components of the senile plaques, may produce reactive oxygen species (ROS) (8), however the exact mechanism of β-amyloid protein in the pathogenesis of dementias of old age is presently unclear.

β-Amyloid may lead to the production of oxidizing agents causing lipid peroxidation (9). Lipid peroxidation has been shown in several studies of patients with AD to be a major cause of membrane dysfunction and subsequent cell death, particularly in the cortex region of the brain (10). There is a clear involvement of free radicals and ROS in AD, but it is not known if free radicals are the primary cause of the neuronal damage that occurs. Even if free radicals are not the specific cause of the disease they are likely to be an essential factor in the pathogenesis of AD and are consequently a potential target for therapy (11). Antioxidants, such as vitamins C and E, may have a therapeutic role in protecting the brain cells from free radical damage (12,13), thereby slowing the rate of decline and improving symptomatology (14). Vitamin E slows down the chain reaction of lipid peroxidation, while vitamin C acts as a scavenger of free radicals in the brain. Lowered serum levels of both vitamin C and vitamin E have previously been reported (15) in patients with AD.

In a study conducted by Zaman et al, patients with both multi-infarct dementia and AD had significantly lower plasma levels of vitamin E and β-carotene than controls (16). Plasma vitamin C levels have been shown to be lower in patients with Alzheimer’s Disease, compared to control subjects, and were
inversely associated with the degree of cognitive impairment. The differences were not explained by lower dietary vitamin C intake in AD patients (12). The authors concluded that their results supported the theory that oxygen free radicals may cause cerebral cortex damage.

Another study in which all subjects with dementia had a normal nutritional status, identified significant deficiencies of plasma antioxidants (17). There is considerable support for the hypothesis that excessive free radical activity occurs in dementia (18) and that supplementation may have a therapeutic role (19).

The present study was conducted to assess whether the plasma vitamin E and C status of subjects with dementia, including Alzheimer’s Disease, differs from elderly subjects with little or no cognitive impairment. To our knowledge, the findings of Riviere and colleagues (12) have not been repeated in other elderly populations and certainly no study of this nature has been conducted in South Africa. The findings will be used to identify appropriate future intervention strategies which may help to delay the progression of cognitive impairment in elderly patients with dementia or Alzheimer’s Disease.

Materials and Methods

Subjects and sampling

A case control study was conducted in 93 subjects aged 65 years and older from two old-age homes in Johannesburg, South Africa. The subjects were either English- or Afrikaans speaking with at least a primary school education. The dementia group (N = 43) included 28 subjects with senile dementia and 15 subjects with diagnosed Alzheimer’s Disease (AD), while the control group included 50 subjects recruited from the same homes with no cognitive impairment. Study subjects were recruited by the matrons or nurses-in-charge at each of the homes. A clinical diagnosis of AD was defined by subjects’ doctors, according to the criteria drawn up by McKhann et al.,1984 (20). No definite diagnosis of AD can be made without an examination of the brain tissue obtained at autopsy or biopsy, therefore the subjects were classified as either “probable” or “possible” cases of AD. Senile dementia was defined according to a clinical diagnosis by the subject’s doctor, as well as a Mini Mental State Examination (MMSE) score of < 24 (21).

Prior to inclusion in the study, written informed consent was obtained from all the control subjects, and from the family members, next-of-kin or power of attorney for those subjects with AD or dementia.

Blood pressure and fasting blood glucose were measured in eligible, consenting subjects in order to exclude subjects with uncontrolled hypertension (blood pressure (BP) ≥ 160/95 mm Hg) and/or uncontrolled diabetes (fasting blood glucose ≥ 7.8 mmol/l) (22), as these conditions have previously been shown to be associated with memory impairment. Blood pressure was measured according to the American Heart Association Recommendations for Human Blood Pressure Determination (23), using automated Omron blood pressure monitors which have been endorsed by the British Hypertension Association as being reliable for use in clinical trials. A large cuff was used for subjects with a mid-arm circumference of ≥ 33 cm. After being seated for at least 5 minutes, three readings were recorded at 1 minute intervals and an average of these 3 readings was calculated. Hypertension was categorized as BP ≥ 140/90 mmHg (24), or if a subject had a BP lower than this cut-off but was previously diagnosed and taking anti-hypertensive medication. Fasting capillary blood glucose was measured using a finger-prick glucometer.

Methods

Blood samples were taken at the fold of the elbow, after 8 hours of fasting, using the vacutainer method. Collections were obtained for vitamin C analyses in a 10 ml heparinized tube and for vitamin E analyses in a 5 ml plastic tube which contained serum gel. Both tubes were placed on ice and delivered to the laboratory within 60 - 90 minutes of being collected and samples were separated by centrifugation. Vitamin C was analyzed immediately and vitamin E samples were stored at -20°C for ± 1 week and then transferred to -70°C for the rest of the storage period, whereafter the samples were analyzed in batches.

Plasma analyses for vitamin C and E

Plasma vitamin C was analyzed using the 2,6-dichlorophenolindophenol titration method, which involves the reduction of a blue dye by ascorbic acid, causing decreased absorption at 520 nm. At a pH of 3 - 4.5 under nitrogen, the dye is not affected by oxidized metabolites of ascorbic acid. The assay measures the reduced form of ascorbic acid (25 - 27).

Plasma vitamin E was determined using a modified version of the reversed-phase HPLC method described by Catignani and Bieri (28). After deproteinization and extraction with ethanol and hexane, the samples were injected onto a C18 - reversed phase separation column. α-Tocopherol (sigma T-3251) was used as an external standard and α-tocopherol acetate (sigma T-3376) as an internal standard. The absorption of α-tocopherol and α-tocopherol acetate were measured at 292 nm and 285 nm respectively, using a programmable UV detector. The coefficient of variation in the laboratory where analyses were performed were 4.5% (intra-assay) and 5.5% (inter-assay) for vitamin E. Plasma vitamin E levels were expressed as a ratio of total plasma lipid (sum of triglycerides and total cholesterol).

Lipid analyses

Analyses of serum total cholesterol and HDL-cholesterol and serum triglycerides were performed using enzymatic assay methods on a technicon RA1000 auto analyser (Bayer Corporation). LDL-cholesterol was calculated using the Friedewald Formula: LDL-chol = [Total chol] - [HDL-chol] - ([TG]/2.2) where all concentrations are given in mmol/L (29).
Dietary assessment

The dietary intake of energy, vitamin C and vitamin E was performed using the plate-waste method (30) repeated for 2 days, one weekday and one weekend day. The quantity of food served at each meal during the day of investigation to each subject was weighed at the point of serving (ie. in the kitchen) using an electronic scale. After the subjects had completed their meal, food wastage on each plate was recorded and actual food intake was calculated in grams. Only food eaten at mealtimes was observed and recorded. The control group were also questioned by a single dietitian (TR) on any food or drink items consumed between meals. Nursing ward staff were asked to report on the between-meal snacks of the cognitively impaired subjects. All recorded food and beverages which were not weighed by the research dietitian were quantified using the Medical Research Council Food Quantities Manual (31). Mean daily food intake for each subject was calculated using the Food Finder Dietary Assessment Computer Package, the database of which is the South African Food Composition Tables (32,33).

Eleven patients (3 AD and 7 dementia subjects) and fourteen control subjects were habitually consuming vitamin C supplements (non significant difference between groups). Two patients (1 AD and 1 dementia subject) and four control subjects were consuming supplements containing vitamin E. The amount of vitamin C and E contained in the supplements was quantified and included in subjects’ assessment of average daily dietary intake. The quantity of vitamin C and E obtained from the food and supplements were together known as the total dietary vitamin C and E intake.

Assessment of nutritional status

Body mass was measured to the nearest 0.01 kg using an electronic scale. Subjects were weighed barefoot, wearing light clothing. Height was measured to the nearest 0.01 cm using a stadiometer, with shoes removed, shoulders relaxed and arms hanging comfortably by the side and head in the Frankfurt horizontal position. Knee height was taken for all subjects and used to estimate the standing height for subjects in wheelchairs where the actual height was unobtainable, using the formula of Chumlea et al (34). The knee and ankle was bent at a 90° angle, and a flat-surfaced object placed on top of the knee while a stiff measuring tape was used to measure the length of the lower leg from the knee to the ankle. Body mass index (BMI) was calculated as mass (kg)/ height squared (m²).

Mid-upper arm circumference was measured at its mid-point, located after bending the elbow at a 90° angle and placing the forearm palm across the trunk. Using a flexible measuring tape, the mid-point of the arm was measured as being halfway between the acromion process of the scapula and the olecranon process (tip) of the elbow (35). The circumference of the arm was measured at the mid-point and recorded to the nearest 0.5 cm.

Calf circumference was measured, as it has been shown to be the most sensitive measure of muscle mass in the elderly (36,37). The largest circumference of the calf was measured in duplicate with the knee and ankle bent to a 90° angle. The measurement was recorded to the nearest 0.5 cm.

All anthropometrical measurements were performed in duplicate and the average of the two measurements taken.

Questionnaires

The Mini Nutritional Assessment (MNA) (38) was used to assess the nutritional status of subjects. The protocol comprises 18 items involving anthropometry, general assessment, dietary assessment, and subjective assessment. MNA has been validated for use in older adults (39,40) and can categorize subjects according to: well-nourished (≥ 24 points); at risk of malnutrition (17 - 23.5 points); or malnourished (< 17 points).

The Barthel Index, a questionnaire on skills/disabilities of activities of daily living (ADL), which consists of 10 questions ranging from bowel and bladder control items to mobility and personal care items, was used to determine the subjects’ level of functional independence (41). The maximum score is 20 points (normal); less than 10 points indicates severe functional impairment.

For subjects suffering from AD or senile dementia, nurses or caregivers who were familiar with the subjects, were asked to complete the various questionnaires.

Cognitive function was determined using Folstein’s Mini-Mental State Examination (MMSE) (42) whereby subjects were asked a number of questions to test their level of cognition, and a maximum score of 30 points was assigned. Some of the subjects were visually impaired and therefore their maximum score was reduced according to the number of questions that they could answer and the overall percentage score was calculated for each subject. Subjects are categorized according to the following groups: no cognitive impairment (≥ 24/30 or ≥ 80 %); mild cognitive impairment (18 - 23 or 60 % - 77 %); severe cognitive impairment (0-17 or 0 %- 57 %) (43).

Data Analysis

Means and standard deviations were used to describe normally distributed continuous variables, while medians and interquartile ranges were used for non-parametric data. Variables were compared for differences between the two groups using the independent t-test for normal data, and the Mann-Whitney test for skewed data.

Correlational analyses between MMSE score and plasma vitamin C and E concentrations were conducted using Pearson correlation coefficients. Multiple regression modelling was performed to investigate the effect of group (ie. dementia status) on plasma vitamin C concentrations, while controlling for potential confounding variables. Due to the non-parametric distribution of plasma vitamin C, the variable was log transformed for the regression analyses.
PLASMA VITAMIN C AND E STATUS OF DEMENTIA PATIENTS

Results

The mean age of the subjects was 84.1 (SD=6.5) years. The dementia group was slightly older than the control group (see Table 1), but this difference did not reach significance (P = 0.069). No difference was found for length of stay in the homes between the groups (mean = 53.3 (58.8) months). Fasting plasma glucose was lower in the dementia group (4.72 (0.83)), compared to the control subjects (5.12 (0.82)) (P<0.05).

Table 1
Subject Characteristics: mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Dementia group</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>43</td>
<td>50</td>
<td>93</td>
</tr>
<tr>
<td>Age (years)</td>
<td>85.4 (6.7)</td>
<td>82.9 (6.2)</td>
<td>84.1 (6.5)</td>
</tr>
<tr>
<td>Proportion of men to women (%)</td>
<td>5:38</td>
<td>4:46</td>
<td>9:84</td>
</tr>
<tr>
<td>(men)</td>
<td>11.6 %</td>
<td>8.0 %</td>
<td>9.7 %</td>
</tr>
<tr>
<td>Length of stay (LOS) (months)</td>
<td>52.8 (65.1)</td>
<td>53.8 (53.6)</td>
<td>53.3 (58.8)</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>4.72 (0.83)</td>
<td>5.12 (0.82)*</td>
<td>4.93 (0.84)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>126 (17.3)</td>
<td>131 (128.8)</td>
<td>129 (152)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74 (13.1)</td>
<td>76 (110.0)</td>
<td>75 (120.0)</td>
</tr>
<tr>
<td>% subjects with hypertension</td>
<td>44.2</td>
<td>64 %†</td>
<td>55%</td>
</tr>
<tr>
<td>ADL Score #</td>
<td>12.5 (10.0)</td>
<td>19.0 (3.0)**</td>
<td>17.0 (6.0)</td>
</tr>
<tr>
<td>MMSE (%)#</td>
<td>43.3 (30.7)</td>
<td>93.2 (17.0)**</td>
<td>76.7 (50)</td>
</tr>
</tbody>
</table>

* P < 0.05; **P < 0.005: Independent t-test for differences between groups. # Median (IQR); Mann-Whitney test between groups. † X² test between groups.

Over half (54 %) of subjects were hypertensive, but all subjects had blood pressure levels < 160/95 mm Hg. The level of functional independence, measured by the ADL score, was lowest in the dementia group. The MMSE score was significantly higher in the control group, indicating a higher level of cognitive impairment in the patient group (Table 1). Median MMSE scores of the dementia group fell within the category of severe cognitive impairment, according to the classification of Tombaugh et al. (43).

Anthropometrical status, using the indicators of body weight, BMI, mid-arm circumference, and calf circumference, were all significantly higher in the control group, indicating a better nutritional status, compared to the dementia group (Table 2). A poorer nutritional status of the dementia group was also reflected in the significantly lower MNA score, compared to the control group (Table 2). Median MNA score fell in the “at risk of malnutrition” category for the dementia group (i.e. 17 - 23.5 points), and in the “well nourished” category for the control group (i.e. ≥ 24 points) (38).

Table 2
Anthropometrical variables and indicators of adequacy of dietary intake, by group

<table>
<thead>
<tr>
<th></th>
<th>Dementia Group</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>43</td>
<td>50</td>
<td>93</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.50 (0.08)</td>
<td>1.50 (0.10)</td>
<td>1.50 (0.09)</td>
</tr>
<tr>
<td>Weight (kg) #</td>
<td>51.0 (10.1)</td>
<td>59.5 (24.7)**</td>
<td>57.2 (18.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4 (4.1)</td>
<td>27.2 (5.3)**</td>
<td>25.5 (5.1)</td>
</tr>
<tr>
<td>Mid-upper arm circumference (cm)</td>
<td>25.6 (4.0)</td>
<td>28.8 (4.1)**</td>
<td>27.3 (4.1)</td>
</tr>
<tr>
<td>Calf Circumference (cm)</td>
<td>31.3 (3.3)</td>
<td>35.5 (4.7)**</td>
<td>33.6 (4.6)</td>
</tr>
<tr>
<td>MNA score #</td>
<td>22.5 (5.5)</td>
<td>25.1 (2.8)**</td>
<td>24.5 (4.0)</td>
</tr>
<tr>
<td>% of subjects with MNA ≤ 23.5 †</td>
<td>62.8 %</td>
<td>26 %**</td>
<td>43 %</td>
</tr>
<tr>
<td>% of subjects with energy intakes &lt; 2/3 of the RDA †</td>
<td>2.3 %</td>
<td>32.0 %**</td>
<td>18.3 %</td>
</tr>
</tbody>
</table>

* P < 0.05; **P < 0.005: Independent t-test for differences between groups. # Median (IQR); Mann-Whitney test for differences between groups. † X² test between groups.

Mean or median concentrations of dietary and plasma vitamin C and E are shown in Table 3.

Table 3
Plasma and Dietary vitamin C and E concentrations of subjects according to group

<table>
<thead>
<tr>
<th></th>
<th>Dementia group</th>
<th>Controls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>43</td>
<td>50</td>
<td>93</td>
</tr>
<tr>
<td>Vitamin C Plasmatic Vitamin C (mg/dl) #</td>
<td>0.560 (0.800)</td>
<td>0.840 (0.540)*</td>
<td>0.795 (0.600)</td>
</tr>
<tr>
<td>Vitamin C Dietetic Vitamin C (mg/day) #</td>
<td>79.5 (151.0)</td>
<td>59.0 (62.0)</td>
<td>67.2 (86.2)</td>
</tr>
<tr>
<td>Vitamin E Plasmatic Vitamin E (mg/dl)</td>
<td>16.66 (4.71)</td>
<td>18.12 (4.48)</td>
<td>17.46 (4.65)</td>
</tr>
<tr>
<td>Vitamin E Dietetic Vitamin E (mg/day) #</td>
<td>2.27 (0.74)</td>
<td>2.32 (0.58)</td>
<td>2.27 (0.64)</td>
</tr>
</tbody>
</table>
| Vitamin C status Median plasma concentrations of vitamin C were significantly lower in dementia subjects, compared to the control subjects (median = 0.56 (IQR = 0.80) and 0.84 (0.54), respectively; P = 0.0398) (See Figure 1). There was no difference in plasma vitamin C levels, according to vitamin C supplement use in either the Dementia (p = 0.117) or the Control (p = 0.698) groups (median = 0.400 (0.230) and 0.645
(0.700) for supplement consumers and non-consumers, respectively, in the Dementia Group, and 0.810 (0.361) and 0.840 (0.640), respectively for the Control group).

**Figure 1**
Median concentrations of plasma vitamin C in dementia subjects and controls

Significantly more dementia subjects had sub-optimal or deficient plasma vitamin C levels, compared to control subjects. Nine percent of subjects with dementia had deficient plasma vitamin C concentrations (< 0.2 mg/dl), while none of the control subjects had concentrations in that range (Figure 2).

**Figure 2**
Categorization of plasma vitamin C status, according to dementia and control groups

The risk of having a suboptimal plasma vitamin C status (≤ 6mg/dl) was almost three times higher in the dementia group, compared to controls (Odds Ratio = 2.99 (95 % CI = 0.95 – 9.79); P = 0.04).

In the total group of subjects, a positive and significant correlation was found between plasma vitamin C concentration and cognitive function (MMSE score) (r = 0.21; P<0.05).

There were no differences between the groups for dietary intake of vitamin C, either from food sources alone or from food and supplement intake. Taking supplement use into account, as well as dietary intake, 42 % of dementia subjects (n = 18) and 43 % of control subjects (n = 22) had inadequate vitamin C intakes (ie. < two-thirds of DRI (44); DRI = 90 mg for men and 75 mg for women). Only 4 subjects (2 from the dementia group and 2 controls) were re-classified as having an adequate vitamin C intake once supplement use was taken into account.

Multiple regression analysis was performed in order to determine predictors of plasma vitamin C concentrations. Controlling for age, sex and total dietary vitamin C intake, a significant group effect was found. In other words, dementia status independently predicted a lower plasma vitamin C status (Table 4). The model was significant, and 6.4% of the variability of plasma vitamin C was explained by these four variables.

**Table 4**
Regression Summary Statistics for Plasma Log Vitamin C Determination

<table>
<thead>
<tr>
<th>B coefficient</th>
<th>Standard error of B</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.4997</td>
<td>1.0468</td>
</tr>
<tr>
<td>Age</td>
<td>-0.0082</td>
<td>0.0101</td>
</tr>
<tr>
<td>Sex</td>
<td>0.1332</td>
<td>0.2115</td>
</tr>
<tr>
<td>Group (Dementia vs Controls)</td>
<td>0.2787</td>
<td>0.1262</td>
</tr>
<tr>
<td>Vitamin C with supplementation</td>
<td>0.0004</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

The model is significant; the adjusted R² = 0.064 (SE = 0.590) (P = 0.0449)

**Vitamin E status**
Plasma vitamin E concentrations (absolute or expressed per total lipid) did not differ significantly between the groups (Table 3). Dietary vitamin E was similar between dementia patients and control subjects. No association was found between plasma vitamin E and MMSE scores.

**Serum lipid concentrations**
Regarding serum lipid concentrations, HDL was the only parameter which differed between the groups (ie. higher in control subjects) (Table 5).

**Table 5**
Lipid concentrations of subjects, according to group: mean (SD)

<table>
<thead>
<tr>
<th></th>
<th>Dementia group</th>
<th>Controls</th>
<th>P value (independent t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>6.39 (1.54)</td>
<td>6.77 (1.34)</td>
<td>0.732</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/l)</td>
<td>1.43 (0.41)</td>
<td>1.71 (0.56)</td>
<td>0.039*</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (mmol/l)</td>
<td>4.37 (1.29)</td>
<td>4.44 (1.21)</td>
<td>0.662</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/l)</td>
<td>1.18 (0.52)</td>
<td>1.27 (0.41)</td>
<td>0.123</td>
</tr>
</tbody>
</table>
PLASMA VITAMIN C AND E STATUS OF DEMENTIA PATIENTS

Discussion

Our findings have demonstrated that older institutionalised adults with either Alzheimer’s Disease or senile dementia have significantly lower plasma vitamin C concentrations than age-matched controls with no cognitive impairment. Similar to the findings of Riviere et al (12), the differences in vitamin C status were not explained by differences in dietary intake between the groups. Plasma vitamin C concentrations were positively correlated with MMSE score, a finding which has previously been shown (12).

It has been demonstrated that even in the healthy elderly, a higher level of plasma vitamin C is associated with better cognitive functioning. A study of 260 healthy elderly aged 60 + found a significant association ($r = 0.15$) between plasma vitamin C concentrations and a memory function test (45), while similar findings have been demonstrated in an elderly Chinese population (46). In the Basel Longitudinal study memory performance was shown to be associated with plasma vitamin C and b-carotene samples taken 22 years earlier (47). A study of 921 community-dwelling elderly people in the United Kingdom reported that cognitive function was poorest in those with the lowest vitamin C status, whether measured by dietary intake or plasma ascorbic acid concentration. The relationship between vitamin C status and cognitive function in that study was independent of age, illness, social class, or other dietary variables (48). La Rue et al (49) have also demonstrated a positive association between vitamin C intakes, plasma levels and cognitive performance in healthy elderly subjects.

However, conflicting evidence has been reported by Perkins et al. in a study of multi-ethnic elderly people in the USA (50). Similarly, analysis of the Rotterdam data at 4 years of follow up did not find an association between cognitive function (MMSE) and baseline dietary intake of vitamin C or E (51). The prospective Zutphen Elderly Study also failed to find an association between antioxidant intake and cognitive functioning (52).

Our finding that plasma vitamin E status (expressed either per unit of total lipids or as absolute values) did not differ between the cognitively impaired subjects and controls is surprising. Vitamin E appears to play an important role in maintaining neuronal integrity and preventing cell loss (53). Vitamin E is the only lipid-soluble, chain-breaking antioxidant found in biological membranes (54); its protective role in neurological conditions where oxidative stress is implicated thus appears promising. An association between a slower rate of cognitive decline and self-supplementation with either vitamin E alone (17) or in conjunction with vitamin C (55, 56) has been found in epidemiological surveys. Morris and colleagues (1998) found a protective effect of vitamin E and C supplementation for incident Alzheimer’s Disease in men (57). However, such studies have been criticized for possible bias and confounding, as habitual consumption of vitamin supplements may simply be a marker for some other protective health related behaviour (58). Nevertheless, stronger evidence is now beginning to emerge from controlled supplementation trials. Oral supplementation with both vitamin E (1 000 mg/day) and vitamin C (300 mg/day) for 12 months improved memory, performance and mood in a healthy elderly population (59).

A 2-year controlled trial of selegiline (a selective monoamine oxidase inhibitor), vitamin E (2 000 IU/day), or both, undertaken in 341 patients with Alzheimer’s Disease showed that there were significant delays in the time to the primary outcome (namely death, institutionalization, loss of the ability to perform basic activities of daily living, or severe dementia), in subjects assigned to the selegiline, vitamin E or combination groups (19). No difference in outcome was found between the selegiline or vitamin E groups, and no additive effect with combined therapy was seen. It is noteworthy that the dosage of vitamin E provided was 133 times higher than the dietary reference intake (44), which is a possibly dangerous level, in terms of overall risk of cardiovascular mortality. The CHAOS study demonstrated an excess (albeit non-significant) of cardiovascular deaths, in patients with known coronary heart disease who were supplemented with vitamin E (either 400 or 800 IU/day) (60). For the moment, the debate regarding safe levels of vitamin E supplementation in middle aged and elderly subjects prohibits widespread use of high-dose supplements.

Evidence regarding the vitamin E status of cognitively impaired older adults is not consistent. Contrary to our findings, other case control studies have found lower plasma levels of vitamin E in subjects with Alzheimer’s disease compared to controls (16, 18). Foy et al have reported a reduction in both plasma vitamin C and E in patients with AD (17). However, the study of Riviere et al (12) found no difference in plasma vitamin E levels between AD patients and controls, in the presence of lowered plasma vitamin C concentrations in the AD patients.

Regarding dietary intake, the cohort Rotterdam study found that at follow-up six years later, baseline dietary intake of vitamin E intake was associated with a decreased risk for incident dementia, as well as for development of AD (61). After adjustment for age, sex, education, and energy intake, for every standard deviation increase in intake, a 17% (OR=0.83; 0.70 - 0.99) and 19% (OR=0.81; 0.66 - 0.99) reduced risk for dementia and Alzheimer’s Disease, respectively, was found.

In our study, subjects with either Alzheimer’s or senile dementia had a poorer nutritional status than controls, regardless of whether anthropometrical measurements or the MNA score were used as indicators. Weight, BMI, mid-upper arm circumference and calf circumference were all significantly lower in the cognitively impaired subjects, while the median MNA score for these subjects fell in the “at-risk” category. Despite evidence of a poorer nutritional status in the dementia group, compared to controls, energy intakes were adequate, and were significantly higher than for the control subjects. Malnutrition is known to be higher in older persons in hospital
or who are institutionalised, compared to their peers living at home (62,63). All subjects in the present study were institutionalized and there was no difference between the groups in length of stay. An explanation for a better energy intake may be that the cognitively impaired subjects are offered greater assistance with eating by nursing and auxiliary staff, as they are significantly more functionally dependent (as determined by lower ADL scores).

A number of limitations in the present study design need to be considered in the interpretation of the findings. We were unable to recruit a larger number of subjects with AD to the dementia group due to the unwillingness of many of the patients’ next-of-kin to give informed consent. In order to realize the sample size, more than one old age home had to be sampled, which may have introduced bias in terms of differences in dietary intake between the homes. Regarding the validity of the dietary assessment method used, the plate-waste method is considered to be a reliable way of objectively assessing intake at mealtimes (30). Meals were served for individual patients in the kitchen by nursing staff who claimed to know the portion size preferences of each of the residents. The presence of the researchers at the time of food portioning may have influenced the staff to provide larger than usual quantities, however this is unlikely to have resulted in misclassification bias since meals for control subjects were also plated by nursing home staff. Despite an attempt to record the consumption of between-meal snacks and beverages, the plate-waste method only accurately measures food items taken at mealtimes. In the case of subjects with Alzheimer’s disease or dementia, ward nursing staff were asked to report between-meal intake which may have resulted in an observer bias.

Previously, it has been assumed that an overall poor nutritional status and insufficient intake of vitamin C and other antioxidants explains the observed lowered plasma levels in patients with Alzheimer’s and other types of dementia (7). Our data supports the alternative theory that the low plasma vitamin C status of patients with dementia is more likely to be a result of an increased oxidative stress resulting from the disease process itself, rather than due to low dietary intakes of the nutrient. In terms of the biological plausibility of the hypothesis that oxidative stress is associated with the development of AD and other dementias of old age, the brain is a good substrate for oxidation; it is a large consumer of oxygen; and polyunsaturated fatty acids, which are a major component of cell membranes, are highly susceptible to lipid peroxidation (64). In addition, there are areas in the brain that are rich in pro-oxidant iron (65), while the brain is low in antioxidant substances which could protect against free radical damage. Berr (2002) provides a comprehensive review of the role of oxidative stress and cognitive impairment in the elderly (66), while the biochemical mechanisms by which vitamin C, specifically, protects proteins from free radical damage are explained by Birlouez-Aragon and Tessier (67). Once vitamin C has quenched and neutralized oxygen radicals, it can be regenerated into its reduced forms, ascorbyl radical (AH) and dehydroascorbic acid through a glutathione and sulfhydryl compound-dependent reductase, allowing it to be active once again. With aging, insufficient reduction of AH leads to increased glycation and oxidation of proteins (67). The altered metabolism and/or excretion of ingested vitamin C was not investigated in the present study.

In the consensus statement on the role of nutrition in aging which resulted from a meeting of scientists held in Stuttgart in November 2000, the expert group agreed that “there are preliminary indicators that a diet rich in vegetables and antioxidants may contribute to cognition and or memory in the elderly” (58). Martin and colleagues (68) argue, however, that few individuals, particularly older adults, are able to meet the recommendation of the US Department of Agriculture and the National Cancer Institute of five servings of fruit and vegetables per day. These authors suggest that supplementation is necessary to augment the dietary intake of vitamins C and E, if the diet is inadequate to supply an “optimal” 200 mg and 100 – 200 mg, respectively per day (68).

Potential confounders which were not considered in the present study include the role of vitamin B12, B6 and folate in hyperhomocysteinaemia, which has been shown to represent a metabolic link in the pathogenesis of old age dementias (69). We attempted to exclude subjects with vascular (multi-infarct) dementia, including those with diagnosed cerebrovascular disease, and uncontrolled hypertension and/or diabetes. No differences between the groups were found for total serum cholesterol concentrations.

Conclusion

The lower plasma vitamin C levels of institutionalised subjects with Alzheimer’s disease or dementia, compared to controls, contributes to the body of evidence which supports the hypothesis that oxidative stress is associated with Alzheimer’s disease and other dementias, and that antioxidant status may play a protective role. Future studies are needed to investigate whether supplementation with vitamin C is able to slow the progression of cognitive decline in patients with AD or dementia, and to identify optimal levels of intake in these patient groups. Vitamin C supplementation below 2000 mg/day is unlikely to pose safety hazards and may potentially offer other nutritional benefits, such as improved immune function, reduced pressure sore risk, and improved wound healing in this group of elderly who are at high nutritional risk.

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