Note

Peripheral Vitamin C Levels in Alzheimer’s Disease: A Cross-Sectional Study

Kazuki IDE, Hiroshi YAMADA, Yohei KAWASAKI, Mie YAMANAKA, Nobuko KAWAKAMI, Yusuke KATSUYAMA, Hidefumi YOSHIDA, Kang KIM, Emi SHIOSAKI, Akihiro SONODA, Keizo UMEGAKI, and Kiyoshi HARADA

1 Department of Drug Evaluation and Informatics, Graduate School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422–8526, Japan
2 Department of Neurology, and 3 Department of Clinical Laboratory Medicine, Shizuoka General Hospital, Shizuoka 420–8527, Japan
4 Information Center, National Institutes of Biomedical Innovation, Health and Nutrition, Tokyo 162–8636, Japan

Summary We previously reported lower lymphocyte vitamin C levels in individuals with type 2 diabetes mellitus and in individuals with severe Parkinson’s disease. Oxidative stress has been proposed to play a key role in the progression of Alzheimer’s disease. Thus, the objective of this study was to investigate the association between peripheral levels of vitamin C and the progression of cognitive dysfunction in Alzheimer’s disease. Fifty individuals with Alzheimer’s disease being treated at Shizuoka General Hospital were consecutively enrolled in this study from December 2009 to March 2015 (76.0 ± 9.7 y of age [mean ± SD]: 32 men and 18 women; Mini-Mental State Examination Japanese version (MMSE-J) score range, 8–27). Plasma and lymphocyte vitamin C levels in fasting blood samples were measured. The association between the MMSE-J scores and vitamin C levels was estimated using Spearman’s rank correlation coefficient (ρ) and the criteria defined by Swinscow. Spearman’s ρ for the relationship between peripheral vitamin C levels and the MMSE-J score was ρ = 0.17 for plasma vitamin C and ρ = 0.26 for lymphocyte vitamin C. Thus, the associations were relatively weak based on the criteria. In contrast with type 2 diabetes mellitus and Parkinson’s disease, lymphocyte vitamin C levels in the peripheral blood may not directly reflect the progression of cognitive dysfunction in Alzheimer’s disease. Additional longitudinal studies are needed to evaluate the clinical importance of changes of peripheral vitamin C status in Alzheimer’s disease.

Key Words cognitive dysfunction, clinical assessment, biomarkers, oxidative stress, vitamin C

Alzheimer’s disease is the most common cause of dementia, accounting for 60–80% of all dementia cases. The number of people living with dementia worldwide in 2010 was estimated to be 35.6 million, and its expected prevalence in 2050 is 115.4 million (1, 2). The cost of care is soaring with the rising number of patients; therefore, it is crucial to identify modifiable risk factors and biomarkers of Alzheimer’s disease in order to develop effective therapeutics and prevention methods (3, 4).

Neuropathologically, Alzheimer’s disease is characterized by an increase of neurofibrillary tangles and amyloid β plaques (5). Additional changes such as brain atrophy, increase of oxidative stress, and neuroinflammation can also occur in the brains of people with Alzheimer’s disease (6, 7). Considering these neuropathological factors, accumulating evidence suggests that oxidative stress may play a key role in Alzheimer’s disease progression (8–10). Various forms of increased oxidative stress have been observed in the Alzheimer’s disease brain, including protein oxidation, lipid peroxidation, DNA and RNA oxidation, and reactive oxygen species formation (9). Several lines of evidence also suggest that amyloid β, which is a trigger protein of Alzheimer’s disease, promotes oxidative stress (8). Oxidative stress is caused by an imbalance between the prooxidant and antioxidant systems. Hence, the status of antioxidant systems may reflect the level of oxidative stress and its consequent effect on disease progression, including Alzheimer’s disease.

Vitamin C is a well-known antioxidant detected in various components of the peripheral blood, and correlations between vitamin C levels in the cerebrospinal fluid and peripheral blood have been reported (11). However, Bowman et al. (11) also reported that plasma vitamin C levels did not independently predict cognitive decline in Alzheimer’s disease. We previously reported...
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Materials and Methods

Participants. Individuals with Alzheimer’s disease who were being treated at the Shizuoka General Hospital were consecutively enrolled in this study from December 2009 to March 2015. Alzheimer’s disease was diagnosed according to the guidelines on disorders associated with dementia (17–20). Computed tomography and magnetic resonance imaging were also used for diagnosis, excluding secondary causes for cognitive dysfunction such as tumor and abscess or normal pressure hydrocephalus.

Written informed consent was obtained from all subjects and/or legally acceptable representatives before enrollment. The study protocol was approved by the ethics committee of the University of Shizuoka (No. 21-3), and Shizuoka General Hospital (No. 21-2) and was conducted in accordance with the Declaration of Helsinki.

Study design. The following clinical characteristics of the participants were recorded: sex, age, body mass index, use of medications for Alzheimer’s disease, duration of Alzheimer’s disease, activity of daily living, complications, smoking habit, alcohol use, and health food consumption. Health food consumption was assessed as the consumption of vitamins and other antioxidant dietary supplements. The progression of cognitive dysfunction was tested using the Mini-Mental State Examination Japanese version (MMSE-J), a well-validated test of cognitive function that is widely used in clinical practice (maximum score, 30). Blood samples were collected under fasting conditions via venipuncture. Plasma and lymphocyte vitamin C levels were measured by high-performance liquid chromatography with an electrochemical detector (HPLC-ECD).

Sample preparation and quantification of plasma and lymphocyte vitamin C. The methods used for sample preparation and quantification of vitamin C were previously described in detail (13). Briefly, plasma and lymphocytes were separated by centrifugation and then immediately treated with metaphosphoric acid (final concentration 5%, wt/wt). Sample preparation was performed on ice within a 2-h time window to obtain reliable data. Prepared samples were stored at −80°C until they were analyzed. Vitamin C levels were measured by the HPLC-ECD method as the total concentrations of ascorbic acid and dehydro ascorbic acid, and all samples were treated in the same manner (12, 21).

Statistical analysis. Associations between peripheral (plasma and lymphocyte) vitamin C levels and the MMSE-J scores were estimated using Spearman’s rank correlation coefficient (ρ) (22), which was interpreted according to the criteria defined by Swinscow (23): 0.00–0.19, very weak; 0.20–0.39, weak; 0.40–0.59, moderate; 0.60–0.79, strong; 0.80–1.00, very strong. Linear regression analysis was used to assess the relationship between peripheral (plasma and lymphocyte) vitamin C concentrations and MMSE-J scores. In this analysis, the peripheral vitamin C concentrations served as the primary predictor variable, and MMSE-J score served as the dependent variable. All statistical procedures were performed using SAS 9.4 for Windows (SAS Institute Inc.; Cary, NC).

Results

Fifty individuals with Alzheimer’s disease (age = 76.0 ± 9.7 y [mean ±SD], age range = 51–97 y; men, n = 32; women, n = 18; duration of Alzheimer’s disease = 2.3 ± 1.9) were enrolled in this study. Among them, 16 individuals had hypertension, 3 had diabetes, and 8 had dyslipidemia. The clinical characteristics of the participants are summarized in Table 1.

<table>
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<th>Clinical characteristics and peripheral vitamin C levels.</th>
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<td>n: 50, Sex: Men (64.0), Women (36.0), Age: 76.0 ± 9.7 y, BMI: 21.2 ± 3.4, MMSE-J score: 19.0 ± 5.2, Medications for AD, n (%): 32 (64.0), Duration of AD, mean ± SD (y): 2.3 ± 1.9, Activities of daily living, n (%): Independent (22.0), Some or full assistance necessary (78.0), Complications, n (%): Hypertension (16.0), Diabetes (3.0), Dyslipidemia (16.0), Smoking, n (%): 9 (18.0), Alcohol use, n (%): 22 (44.0), Health food, n (%): 13 (26.0), Vitamin C levels, mean ± SD [min, max]: Plasma (μmol/L): 38.6 ± 20.1 [2.8, 77.9]. Lymphocyte (nmol/mg protein): 17.2 ± 5.4 [3.2, 27.7].</td>
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Table 1. Clinical characteristics and peripheral vitamin C levels.
and MMSE-J scores were considered or controlled for in the data analysis, owing to the limited number of participants in this exploratory study. Several risk factors of Alzheimer’s disease have been identified, such as aging (2), and large-scale studies considering risk factors are needed. Consideration of lifestyle factors is also important. For example, older patients with Parkinson’s disease (13) and in patients with type 2 diabetes mellitus compared to healthy controls (12); however, in Alzheimer’s disease, other pathophysiological factors might affect peripheral vitamin C status. In fact, several studies have demonstrated lower plasma vitamin C levels in individuals with Alzheimer’s disease compared with healthy controls (26–29), whereas other studies did not find such an association (30–33). These conflicting findings in previous investigations further suggest that there are other pathophysiological factors affecting vitamin C status in Alzheimer’s disease. However, these studies only focused on plasma vitamin C levels, and not on vitamin C levels in the lymphocytes or other components of the blood. In particular, lymphocyte vitamin C levels tend to be unaffected by transient food consumption and circadian rhythms (34), and their concentration is 80 to 100 times higher than that in the plasma (35). The results of this study also support the advantages of conducting a lymphocyte vitamin C assay. The dispersion of vitamin C levels in the lymphocytes was lower than that in the plasma, and Spearman’s rank correlation coefficient (r) for the lymphocyte vitamin C level and MMSE-J score was stronger than that for the plasma vitamin C level. Therefore, future studies focusing on lymphocyte vitamin C status may provide more reliable results with respect to changes in disease onset and progression.

For future studies, assuring the reliability and accuracy of the methods is also an important consideration. In terms of the vitamin C assay, sample preparation in this study was performed on ice within a 2-h time window. Prepared samples were stored at −80°C until they were analyzed using the HPLC-ECD method, and all samples were treated in the same manner (12, 21). This well-controlled procedure guaranteed the reliability and accuracy of the method. The concentrations of peripheral vitamin C are also affected by the individual’s medical condition; therefore, it is difficult to compare our results directly with those of previous studies. However, the dispersion of lymphocyte vitamin C was lower than that of the plasma, even after considering the results of previous studies. This also supports the usefulness of conducting a lymphocyte vitamin C assay for evaluating oxidative stress status. The progression of the cognitive dysfunction of the participants was tested using the MMSE-J. This is a well-validated test that is widely used in clinical practice; in addition, the participants were assessed by a physician at the Department of Neurology in the hospital, who was usually a specialist in neurology. This protocol supported the accuracy of the assessment of cognitive function.

However, there are several limitations to this study that should be pointed out. The main limitation is the potential for confounding factors that were not considered or controlled for in the data analysis, owing to the limited number of participants in this exploratory study. Several risk factors of Alzheimer’s disease have been identified, such as aging (2), and large-scale studies considering risk factors are needed. Consideration of lifestyle factors is also important. For example, older

Fig. 1. Correlation between plasma (A) and lymphocyte (B) vitamin C levels and MMSE-J scores.

Among the 50 participants, 13 participants habitually consumed health food (dietary supplements), 22 participants had a drinking habit, 9 participants had a smoking history, and 32 participants had been treated with medications for Alzheimer’s disease. The range of MMSE-J scores was 8–27. All blood samples obtained from the 50 participants were analyzed, and mean±SD peripheral plasma and lymphocyte vitamin C levels were 38.6±20.1 μmol/L and 17.2±5.4 nmol/mg protein, respectively. The Spearman’s rank correlation coefficients between plasma and lymphocyte vitamin C levels and MMSE-J scores were ρ=0.17 and ρ=0.26, respectively (Fig. 1).

Therefore, the associations between MMSE-J score and peripheral vitamin C concentration were considered to be “very weak” in the plasma and lymphocytes among these subjects with Alzheimer’s disease based on the criteria defined by Swinscow (23).

Discussion

In this study, peripheral vitamin C levels were weakly correlated with the severity of cognitive dysfunction in Alzheimer’s disease according to the MMSE-J score based on the criteria defined by Swinscow (23). Although there was no strong correlation, the plasma vitamin C levels in 36.0% of the individuals that participated in this study were under 30 μmol/L (24, 25), which is considered to represent vitamin C depletion according to clinical standards, and thus may have a greater impact on disease progression over time.

We previously reported that lower lymphocyte vitamin C levels were associated with more severe forms of Alzheimer’s disease according to the MMSE-J score based on the criteria defined by Swinscow (23). Although there was no strong correlation, the plasma vitamin C levels in 36.0% of the individuals that participated in this study were under 30 μmol/L (24, 25), which is considered to represent vitamin C depletion according to clinical standards, and thus may have a greater impact on disease progression over time.
individuals with poorer cognitive function may be more likely to have an attentive caretaker who is vigilant about the types of food consumed and the dietary supplements they take, whereas younger individuals with higher cognitive function may not be as vigilant about their diet and dietary supplements, and thus might have a relatively low vitamin C capacity. In addition, there may be complex relationships between redox status and disease progression; thus, the evaluation of other compounds such as markers of oxidative stress or other clinical parameters may provide clarity and new information about this association. Another limitation of the present study is the cross-sectional design, which might make it difficult to reach a meaningful conclusion about the relationship between peripheral vitamin C and cognitive decline. Alzheimer’s disease is a progressive neurodegenerative disorder (36); thus, longitudinal observation is essential to best understand the relationship between the progression of cognitive dysfunction in Alzheimer’s disease and peripheral vitamin C status.

When conducting additional longitudinal studies, a study design considering confounding variables, and comparisons between healthy control subjects or patients with other neurodegenerative disorders will also be important. We performed an exploratory analysis to compare the difference in vitamin C concentrations according to sex and drugs used for Alzheimer’s disease. The plasma and lymphocyte vitamin C concentrations were not different between groups (Wilcoxon rank-sum test, p>0.05), suggesting that these factors may not strongly affect the peripheral vitamin C concentration in individuals with Alzheimer’s disease. The results indicated that the feasibility of an additional longitudinal study could be relatively high. In these future studies, comparison with healthy adults and/or patients with other neurodegenerative disorders would provide important information on the value of peripheral vitamin C levels as a disease marker, and would allow for better estimations of the difference in vitamin C levels in diseased or healthy status.

Conclusions

In contrast with previous studies of patients with type 2 diabetes mellitus and Parkinson’s disease, our results suggest that plasma and lymphocyte vitamin C levels may not directly reflect the progression of cognitive dysfunction in Alzheimer’s disease. However, further investigations considering physiological and pathological changes of patients with Alzheimer’s disease, as well as longitudinal studies, are required to more comprehensively evaluate the clinical importance of changes in peripheral vitamin C status in Alzheimer’s disease.

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Author contributions

H. Yamada, KI, KH conceived and designed the study. NK, Y. Katsuyma, H. Yoshida, KK, ES, AS, KH, KI collected and managed the data. KI, Y. Kawasaki, MY, H. Yamada, KU analyzed and interpreted the data. KI wrote and revised the manuscript.

REFERENCES


