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ORIGINAL ARTICLE

Raised homocysteine and low folate and vitamin B-12 concentrations predict cognitive decline in community-dwelling older Japanese adults

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Summary
Background & aims: Recently, poor cognition and dementia have been associated with elevated homocysteine and low B vitamin concentrations. The aim of this study is to examine the association in community-dwelling older Japanese adults.
Methods: Ninety-nine subjects (71 women and 28 men; mean age 75 years) were eligible for analysis after exclusion of subjects with high serum creatinine concentrations (1.3 mg/dl and over) and those taking vitamin supplements. Fasting blood samples were analyzed for plasma total homocysteine, serum folate, and serum vitamin B-12. Global cognitive function was assessed using the Mini-Mental State Examination (MMSE).
Results: Multiple regression analysis revealed that homocysteine concentrations were predicted by concentrations of vitamin B-12 ($p < 0.001$), folate ($p < 0.005$), and creatinine ($p < 0.001$) and age ($p < 0.005$). Scores on the MMSE were associated with concentrations of homocysteine, vitamin B-12, and folate. The association between folate or vitamin B-12 concentrations and MMSE scores remained significant after adjusting for homocysteine concentrations. Folate concentrations, but neither homocysteine nor vitamin B-12 concentrations, were significantly associated with serum albumin concentrations.

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Conclusions: Reduced folate and vitamin B-12 concentrations were independently associated with cognitive decline. The correlation between folate and albumin concentrations may imply that the reduction of folate in the Japanese older population is due to nutritional deficiency.

Introduction

As life expectancy almost doubled in the 20th century, the numbers of frail elderly persons with cognitive decline or dementia have continued to increase in the developed countries. Despite recent striking advances in understanding the pathogenesis of dementia, most dementing illnesses are irreversible and involve progressive impairment. The onset of dementia leads to deterioration in the quality of life and loss of independence for patients. Therefore, the discovery of measures to prevent cognitive decline and dementia is crucial.

Homocysteine is an amino acid formed during the metabolism of methionine. Major factors determining plasma total homocysteine (tHcy) concentrations in the general population include age, vitamin concentrations (folate, vitamins B-6, and B-12), renal function, methylene tetra-hydrofolate reductase (MTHFR) polymorphism (677 C → T), and drugs.[1] Elevated homocysteine is an independent risk factor for atherosclerosis.[2,3]

Recently, several lines of evidence have indicated that increased homocysteine concentrations are associated with cognitive decline and dementia.[4–6] A recent report has indicated that high homocysteine concentrations are a strong and independent risk factor for development of dementia and Alzheimer's disease,[4] although this association remains controversial. Because substantial evidence indicates pathologic interaction between Alzheimer's disease and vascular dementia, particularly in older patients,[7] vascular risk factors such as elevated homocysteine can be involved in the causation of both conditions. Alternatively, homocysteine has been reported to promote apoptosis of neurons[8] or to cause a neurotoxic response,[9] suggesting direct toxic effects on neuronal cells.

In addition to the role for homocysteine elevation in cognition, the contribution of decreased serum folate or vitamin B-12 concentrations remains to be explained.[10–12] Several lines of evidence from recent studies have supported the relative importance of folate over homocysteine for the development of dementia.[13,14]

The mandatory folate fortification program in the United States has been reported to be effective in improving folate and tHcys status in the population of middle-aged and older adults.[15] Folate fortification program has not been instituted in Japan, where the diet, which is rich in vegetables, plants, and beans, has been assumed to contain sufficient folate. We therefore set up this study among community-dwelling Japanese older persons for the following purposes: (1) to examine the concentrations of plasma tHcy and its prediction factors and (2) to examine the relationship between homocysteine and B vitamin concentrations and cognitive function.

Materials and methods

Subjects

Subjects (41 males and 83 females) were recruited from the geriatric clinic and the geriatric wards of Nagoya University Hospital. Written, informed consent was obtained from all study participants. All the subjects were community-dwelling older adults. Although their educational backgrounds are various, most of them had completed at least approximately 9 years of mandatory education. Occupational histories of male subjects are various. In contrast, majority of female subjects had been full-time housewives. They lived at home independently or with a mild decline in activities of daily living. Although many of them were multimorbid, they usually had typical eating habits of community-dwelling older Japanese adults; their diet is rich in vegetables, plants, and beans; they take protein from fish and meat. The subjects were fairly nourished compared with more frail elderly or institutionalized elderly, which was supported by their fairly high albumin levels. Subjects with chronic conditions were taking medicines including antihypertensive drugs such as ACE inhibitors, angiotensin receptor blockers, and Ca channel blockers, anti-platelet agents such as baby aspirin, and antidiabetic drugs. Patients recruited from the hospital wards were not in an acutely ill condition and had mostly been admitted for physical or mental evaluation. To be eligible for the analysis, subjects had to be aged 60 years or older, not taking any vitamin supplements, and free from overt kidney dysfunction (creatinine concentration 1.2 mg/dl or below); 99 subjects who met these conditions (28 men and 71 women, mean age $\pm$ SD, 75.4 $\pm$ 8.1; range 60–94 years) were included in the analysis.

Anthropometric measurements and biochemical markers

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Blood samples were collected after an overnight fast. Blood samples from hospitalized patients were taken on the day after admission. Plasma homocysteine and serum folate and vitamin B-12 were measured with high performance liquid chromatography (HPLC) (BML, INC, Tokyo) and chemiluminescent immunoassay (CLIA), respectively. Creatinine and cholesterol, albumin, and hemoglobin were measured with the enzyme method, the bromcresol purple (BCP) colorimetric method, and the sodium lauryl sulfate—hemoglobin (SLS—Hb) method, respectively, at the laboratory of Nagoya University. All blood draws were performed by medical laboratory technicians in an ambulatory setting or nurses in an inpatient setting at Nagoya University Hospital, and the blood samples were collected in designated tubes and handled properly. Plasma samples were collected in tubes with anticoagulants, mixed promptly, and centrifuged immediately.

Evaluation of cognitive function and depression

The Mini-Mental State Examination (MMSE) was performed for the evaluation of cognitive function.[16] The maximum score is 30 points, and a score of 23 or less is generally considered suspicious for cognitive impairment. The Zung Self-rating Depression Scale was used to measure depressive symptoms.[17] A score of 50 points or above indicates a high likelihood of depression. MMSE and the Zung Self-rating Depression Scale were performed by examiners who did not know the results of clinical chemical tests.

Statistical analysis

All statistical tests were two-sided, with $p < 0.05$ as the criterion of statistical significance. The Kolmogorov—Smirnov test was used to determine the normality of data. Logarithmic transformation was used to normalize data with skewed distributions (serum vitamin B-12 and folate and plasma tHcys). The multiple regression method was used to adjust the variables predicting the concentrations of tHcys or the MMSE scores. SPSS 11.0J software (SPSS Inc., Chicago, IL, USA) was used for the data evaluation.

Results

Demographic, clinical, and biochemical characteristics of the subjects

One hundred and twenty-four persons aged 60 years or older (41 males and 83 females) were initially evaluated. Plasma tHcys concentrations were markedly higher (mean plasma tHcys $\pm$ SD, 25.2 $\pm$ 11.9 μmol/L; range 12.1—41.4) among 8 subjects with serum creatinine 1.3 mg/dl and higher (mean serum creatinine $\pm$ SD, 2.1 $\pm$ 1.2 mg/dl; range 1.3—4.9). Thirteen and 4 subjects were taking vitamin B-12 (mecobalamin) and multivitamin supplements, respectively. Plasma tHcys concentrations among subjects taking these supplements were significantly lower than those among subjects in the present analysis (mean plasma tHcys $\pm$ SD, 8.3 $\pm$ 1.8 μmol/L, $p < 0.05$). Twenty-eight men and 71 women aged 60 years and older without overt renal dysfunction (creatinine 1.2 mg/dl or lower) and not taking any vitamin supplements were eligible for the following analysis.

The profiles of subjects are shown in Table 1. Thirty-one subjects (31.3%) had MMSE scores of 23 points or less, indicating cognitive decline. Ten subjects (10.9%) had Zung depression scale scores of 50 or more, indicating depression. The Zung depression scale could not be performed in 8

Table 1 Demographic, clinical, and biochemical characteristics

Demographic characteristics	
Age (years), means $\pm$ SD (range)	75.4 $\pm$ 8.1 (60—94)
Men/women (*n*)	28/71
Clinical characteristics	
MMSE score (range 0—30), means $\pm$ SD (range)	24.6 $\pm$ 5.3 (4—30)
Zung depression score (range 20—80), means $\pm$ SD (range)	37.8 $\pm$ 10.1 (23—77)
Current smokers, % of total	8.1
Diabetes, % of total	34.3
Hypertension, % of total	38.3
Ischemic heart disease, % of total	12.1
Cerebrovascular accident, % of total	16.2
H2-receptor antagonists or proton-pump inhibitor, % of total	16.2
Current alcohol use, % of total	14.1
Body mass index (BMI, kg/m^2), means $\pm$ SD (range)	22.3 $\pm$ 3.8 (13.5—40.4)
Biochemical characteristics	
Total cholesterol (mg/dl), means $\pm$ SD (range)	205.6 $\pm$ 36.0 (107—312)
Total homocysteine (μmol/L), means $\pm$ SD (range)	11.0 $\pm$ 4.9 (4.4—36.4)
Total homocysteine $>$ 14.0 μmol/L, % of total	18.2
Folate (ng/ml), means $\pm$ SD (range)	10.1 $\pm$ 4.6 (2.5—20.6)
Vitamin B-12 (pg/ml), means $\pm$ SD (range)	635.4 $\pm$ 497.8 (202—3090)
Creatinine (mg/dl), means $\pm$ SD (range)	0.746 $\pm$ 0.17 (0.4—1.2)
Albumin (g/dl), means $\pm$ SD (range)	4.1 $\pm$ 0.4 (2.8—4.8)
Lymphocyte count (/μl), means $\pm$ SD (range)	1744 $\pm$ 664 (600—3990)
Hemoglobin (g/dl), means $\pm$ SD (range)	12.6 $\pm$ 1.5 (8.7—16.0)

Table 2 Association between variables

	B-12	Folate	Creatinine	BMI	Hemoglobin
Homocysteine	−0.443*	−0.298**	0.433*	0.151	−0.295**
Vitamin B-12		0.178	0.062	−0.048	0.202
Folate			0.071	0.006	0.350**
Creatinine				0.192***	−0.103
BMI					0.145

Values are standard regression coefficients and adjusted for sex, age, in the presence or absence of smoking, alcohol intake, and diabetes.
*$p < 0.001$, **$p < 0.005$, and ***$p < 0.05$.

subjects with moderate to severe cognitive decline. Only 5 subjects had albumin concentrations of 3.5 g/dl or less. Zero and 4 subjects had serum folate and vitamin B-12 concentrations, respectively, lower than the low end of the reference range (2.4 ng/ml for serum folate; 233 pg/ml for serum vitamin B-12). Many subjects had chronic diseases (e.g., diabetes; 34.3%); however, they lived at home independently or with mild decline in activities of daily living; they ate meals without supplement use.

Predictors of homocysteine concentrations

Plasma tHcys concentrations in our subjects were distributed in a range comparable to those previously reported from the U.S. or Europe. A multiple regression analysis revealed significant inverse associations between tHcys and vitamin B-12, folate, and hemoglobin, and a positive association between tHcys and creatinine (1.2 mg/dl or less), after controlling for sex, age, smoking, alcohol intake, and diabetes (Table 2). Furthermore, a multiple regression analysis was performed using tHcys concentration as the dependent variable and age, creatinine, vitamin B-12, folate, hemoglobin, sex, the presence or absence of smoking, alcohol intake, and diabetes as independent variables. Based on Cook's distance, one subject was excluded as an outlier in the analyses in Table 2 and the multiple regression analysis. Age (standard regression coefficient ± SE, 0.224 ± 0.074, $p < 0.005$), creatinine (1.2 mg/dl or less) (0.461 ± 0.082, $p < 0.001$), vitamin B-12 (-0.409 ± 0.068, $p < 0.001$), and folate (-0.218 ± 0.073, $p < 0.005$) were shown to be independently significant variables. The standard regression coefficient indicated that creatinine concentrations (even within the normal range) and vitamin B-12 concentrations were the principal predictors of homocysteine concentrations.

Cognitive function and concentrations of homocysteine, folate, and vitamin B-12

The inverse or positive associations between the MMSE scores and the concentrations of homocysteine, folate, or vitamin B-12 were shown to be significant after adjusting for sex, age, BMI, creatinine, smoking, alcohol intake, and diabetes, whereas the Zung depression scores did not correlate with any of these variables (Table 3). There was no significant association between the MMSE and the Zung score (Spearman's correlation coefficient, 0.048; $p = 0.648$). The multiple regression analysis using MMSE scores as the dependent variable and folate and vitamin B-12 as independent variables showed they were independently associated with the MMSE scores after adjusting for age, sex, BMI, creatinine, presence or absence of diabetes, smoking, and alcohol intake (Table 4). After adjusting for tHcys, the association between the MMSE score and the concentrations of folate or vitamin B-12 remained statistically significant (Table 3).

Correlation between folate and nutritional variables

Finally, associations between folate or vitamin B-12 and nutritional markers were examined in order to understand what caused the decline of folate or vitamin B-12

Table 3 Association between scores of the Mini-Mental State Examination or the Zung Self-rating Depression Scale and each of the variable

	MMSE ($n = 99$)				Zung ($n = 91$)	
	Model 1[b]		Model 2[c]		Model 1[b]	
	β^a	p	β^a	p	β^a	p
Homocysteine	−0.292	0.011	NA	NA	0.086	0.483
Vitamin B-12	0.289	0.001	0.230	0.034	0.171	0.101
Folate	0.334	0.000	0.282	0.005	−0.084	0.443

NA: not applicable.
[a] Standard regression coefficient.
[b] Values are adjusted for sex, age, creatinine, BMI, and in the presence or absence of smoking, alcohol intake, and diabetes.
[c] Values are adjusted for variables in model 1 + homocysteine.

Table 4 Predictors of MMSE score

	β[a]	p
Vitamin B-12	0.241 ± 0.085	0.006
Folate	0.291 ± 0.088	0.001
Age	-0.211 ± 0.095	0.029

[a] Standard regression coefficient. Values are $x \pm$ SE. Values are adjusted for sex, creatinine, BMI, diabetes, smoking, alcohol intake, and all other variables listed.

concentrations in the subjects. After controlling for sex, age, creatinine, smoking, alcohol intake, diabetes, and MMSE scores, the concentrations of folate, but not those of tHcys or vitamin B-12, were significantly correlated with serum albumin concentration ($p < 0.001$). Lymphocyte counts, BMI, and total cholesterol concentrations did not correlate with concentrations of folate, vitamin B-12, or tHcys (data not shown). There was a significant correlation between concentrations of serum albumin and total cholesterol ($p < 0.001$), but not lymphocyte counts ($p = 0.352$) or BMI ($p = 0.124$).

Discussion

The present study revealed that vitamin B-12, folate, mild renal impairment, and aging are the major predictors of plasma tHcys concentrations in community-dwelling older Japanese people. In addition, we showed that cognitive function was associated with the concentrations of vitamin B-12 or folate. These associations were independent of homocysteine concentrations. The decrease of folate concentrations might be derived from nutritional deficiency in this group, considering that it was correlated with albumin concentrations. To our knowledge, there have been few reports demonstrating a relation between cognitive function and homocysteine, vitamin B-12, or folate in Japanese elderly people, who differ from those in the U.S. and Europe in ethnicity, culture, lifestyles, and food habits. Our data suggesting independent involvement of folate and vitamin B-12 in the cognition of the study group raise the question whether folate and/or vitamin B-12 supplementation is necessary in Japan, where a folate fortification program has not been implemented.

The means and distributions of plasma tHcys in our subjects are comparable to those reported previously from western countries.[1] The predictors of its concentration were found to be age and folate, vitamin B-12, and creatinine concentrations, consistent with previous reports.[1] It should be noted that our subjects included only those with creatinine concentrations of 1.2 mg/dl or less, indicating that even mild reduction of glomerular filtration rate (GFR) is an equivalent or even stronger determinant of homocysteine concentration than age or folate or vitamin B-12 concentration among older persons. It has been reported that GFR is a significant predictor of plasma tHcy concentration in patients with diabetes mellitus and with serum creatinine concentrations less than 1.3 mg/dl.[18] In this previous report, age was not a significant predictor for tHcys concentrations when adjusted by GFR, but in our analysis, age remained significant after adjusting for creatinine concentrations, suggesting the contribution of additional age-related factors.

Several lines of evidence from cross-sectional studies have suggested an association between concentrations of tHcys and cognitive function[5,6] and Alzheimer's disease.[19,20] In addition, brain atrophy has been reported to be associated with increased tHcys concentrations.[21] Notably, Seshadri et al. have demonstrated a causal relation of tHcys increase to dementia and Alzheimer's disease.[4] However, beneficial effects of vitamin B-12 or folate administration, which reliably reduces plasma tHcys concentrations, on cognitive function have not been shown.[22–24] Although supplementation with B vitamins has been believed to be safe, recent interventional studies for cardiovascular disease have revealed no benefits or even potentially harmful effects of B vitamin supplementation on cardiovascular events.[25,26] These unexpected results have been speculated to be caused by differences in the dose and the length of supplementation among studies and have revealed that it is too simplistic to consider the mere reduction of plasma tHcys concentrations as a beneficial change.

It has been reported that mood disorders can be affected by concentrations of tHcys, folate, or vitamin B-12.[27,28] Mood disorders or depression can affect cognitive function. In the present study, however, we did not find any correlation between scores on the depression scale and concentrations of tHcys, folate, or vitamin B-12. Among the subjects in the present study, only 11% of patients had scores of 50 and above, which indicate depression. These patients had lower folate concentrations compared with patients with scores of less than 50 (8.9 vs 10.6 ng/ml). This difference, however, did not reach statistical significance. We think this is probably due to fewer numbers of our subjects with suspected depression. Thus, the association between folate concentrations and depression may exist among community-dwelling older Japanese. Nevertheless, no significant association of the Zung score with MMSE scores or concentrations of tHcys, folate, or vitamin B-12 in the present study suggests that the correlations between MMSE scores and concentrations of tHcys, folate, or vitamin B-12 are not likely to be due to mood disorders.

The association between increased tHcys concentrations and cognitive decline may be attributed to the coexisting folate and/or vitamin B-12 deficiency, at least in part. Notably, recent studies have reported the contribution of decreased folate to cognitive decline in subjects with increased tHcys.[13,14] Furthermore, vitamin B-12 has been reported to be associated with cognition although it remains to be concluded whether vitamin B-12 alone is a causative factor for cognitive decline in the presence of increased levels of tHcys.[29] Our analysis demonstrated an independent association of MMSE scores with folate or vitamin B-12 concentrations after controlling tHcys concentrations, suggesting folate and vitamin B-12 have roles distinct from those of tHcys on the cognition of community-dwelling Japanese older people. Measurement of methylmalonic or holotranscobalamin II which has been shown to be more sensitive for vitamin B-12 deficiency than serum vitamin B-12 concentrations, could clarify the role for vitamin B-12 in cognition.[29,30]

In the present subjects, the concentrations of folate, but not homocysteine or vitamin B-12, had a significant association with serum albumin concentrations, a widely used nutritional parameter.[31] Because total cholesterol, BMI, or lymphocyte counts, other commonly used parameters for nutritional status, did not show significant associations with folate, we think the low serum folate concentrations may be derived, at least in part, from early nutritional deficiency, which only leads to a mild decrease in serum albumin concentrations.

In addition to the small number of subjects, our study has several potential limitations. Because we recruited subjects from among the patients of a university hospital, many had chronic diseases. A further study focusing on community-dwelling disease-free robust elderly people is necessary. We do not know the MTHFR mutation (677 C → T) in our subjects, which has been reported to exist in about 10% of Japanese people.[32]

In conclusion, we assume folate and/or vitamin B-12 supplementation, which would be useful to improve tHcys, folate, and vitamin B-12 status, might reduce the risk of developing cognitive decline in the Japanese elderly population. Although randomized trials using B vitamin have failed to show beneficial effects on cognition of elderly persons,[24] different protocols (i.e. dose and length of B vitamins administration; targeting certain population) need to be tested before denying the usefulness. Moreover it is conceivable that keeping appropriate levels of B vitamins and homocysteine throughout life are useful to prevent cognitive decline in later life. From this standpoint, the appropriate supplementation of B vitamins needs to be considered as the relevant issue for all age groups in Japan.

Conflict of interest statement

The authors have no conflict of interest regarding this project.

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