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Relationship between Decline in Cognitive Function and Homocysteine, Folate, and Vitamin B_{12}

Research Article

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Summary

Hyperhomocysteinemia (HHcy) causes various diseases, including cardiovascular disease, osteoporotic fractures and dementia. Although there have been reports that HHcy impairs cognitive function, findings are inconsistent on the association of homocysteine (Hcy), folate, and vitamin B_{12} with cognitive function. Considering that a lower-quality diet increases the risk of dementia in elderly individuals, the effect of nutritional status must be clarified. In the present study, a case–control study and an intervention study were conducted. In Study 1, 85 patients who visited the clinic with complaints of memory impairment were evaluated for their plasma Hcy, serum folate, serum vitamin B_{12} concentrations and cognitive function. Although not statistically significant, plasma Hcy concentrations in dementia patients (11.00 ± 4.90 nmol/mL) as well as MCI patients (10.7 ± 2.49 nmol/mL) were higher than those in the normal cognitive-function group (9.74 ± 3.84 nmol/mL). In Study 2, 42 patients with HHcy at the first visit were administered Memorin®, a dietary supplement containing vitamin B_{12}, folate, vitamin B_6, and other ingredients, for 10 months. HHcy (12.3 ± 4.58 nmol/mL) was significantly improved 4 months (10.0 ± 3.85 nmol/mL) and 10 months (10.16 ± 3.12 nmol/mL) after the administration of Memorin®. The pharmacological actions of dietary supplements for HHcy were discussed in this article.

Keywords: Folate, Vitamin B_{12}, Homocysteine, Cognitive Function, Dementia, MCI.

Abbreviations

HHcy: Hyperhomocysteinemia; Hcy: Homocysteine; MCI: Mild Cognitive Impairment; AD: Alzheimer's Disease; VaD: Vascular Dementia; ANOVA: Analysis of Variance; TG: Triglyceride; CRISIS: Coronary Risk of Insulin Sensitivity in Indian Subjects study; THF: Tetrahydrofolate; MTHFR: Methylenetetrahydrofolate Reductase.

Introduction

The prevalence and economic costs of Alzheimer's disease (AD) and other types of dementia are increasing along with the increasing elderly population [1]. It is therefore important to identify the modifiable risk factors for dementia [2]. Mild cognitive impairment (MCI) is an intermediate stage in the neurodegenerative pathology from normal brain aging to dementia [2]. Elderly individuals with MCI are at high risk of developing dementia, including AD and vascular dementia (VaD). Subjects with a diagnosis of MCI constitute a clinical entity that can be subjected to preventive measures [2]. B vitamins such as folate, vitamin B_2, vitamin B_6, and vitamin B_{12} are involved in one-carbon

transfer reactions such as methylation, which is necessary for the production of monoamine neurotransmitters, phospholipids, and nucleotides in the brain [3,4]. Low concentrations of these B vitamins have been associated with HHcy [5,6], which is neurotoxic [7]. Moreover, several cross-sectional and longitudinal studies have indicated that HHcy is an independent risk factor for impaired cognitive function [8-11], although other studies found no significant association between Hcy and cognitive function [12,13].

For HHcy, various vitamin-B complex supplements are available to date. However, absorption of vitamin B_{12} from gastrointestinal mucosa is often impaired in the elderly population [14], and the appropriate duration and amount of vitamin B supplementation required for the improvement of HHcy in elderly patients is not well defined. In the current study, the efficacy of Memorin®, a commercially available vitamin-B complex supplement for HHcy in elderly individuals, was investigated.

Materials and Methods

Subjects and study design

Study 1 was a case–control study conducted with 85 patients who visited the Ochanomizu Health & Longevity Clinic from May 2018 to February 2023. Written consent to participate in this study was obtained from each subject after explanation of the objective and protocol of this study. Subjects were excluded if they were taking vitamin B_{12}, folate, or vitamin B_6 at the time of enrollment.

Study 2 was an intervention study conducted from June 2020 to February 2023 for 42 patients with hyperhomocysteinemia from Study 1. The dietary supplement Memorin® was administered orally once a day for 10 months. Blood chemistry and Mini-Mental State Examination (MMSE) were done before, 4 months, and 10 months after the administration of Memorin®.

Dietary supplementation

Memorin® (Lequio Pharma Co. Ltd., https://www.lequio-pha.co.jp/en/index.html) containing 50 mg curcuminoids, 55 mg DHA, 10 mg squalene, 5 mg turmeric powder, 2 mg piperine, 10 mg vitamin B_2, 12.5 mg vitamin B_6, 250 µg vitamin B_{12}, 400 µg folic acid, 50 mg vitamin C, 25 mg vitamin E, 3.2 mg vitamin B_1, and 13 µg huperzine A in one capsule.

Cognitive evaluations

Cognitive evaluations were performed with the Mini-Mental State Examination (MMSE). The MMSE is a well-validated screening tool for global cognitive impairment and dementia in clinical settings [15]. It evaluates multiple cognitive domains, including attention, memory, and executive function. Patients with an MMSE score ≥27 points were categorized as having normal cognitive function, those with a score between 23 and 26 points were categorized as having mild cognitive impairment (MCI), and those with a score <23 points were categorized as having dementia.

Statistical analyses

Statistical analyses were performed using JMP version 17.0.0 (SAS Institute Japan, Tokyo). The difference between men and women was analyzed by Student's t test. Comparisons of subjects' characteristics among normal, MCI, and dementia were carried out using one-way ANOVA followed by Tukey–Kramer multiple comparison tests. Spearman's correlation was used to examine the relationship between plasma Hcy and serum folate, vitamin B_{12}, creatinine concentrations, eGFR, and other biomarkers. Repeated measures ANOVA was used to analyze the effects of Memorin® on Hcy, LDL, TG, vitamin B_{12}, serum folate concentration and MMSE score.

Molecular illustration

The 3D chemical structures of tetrahydrofolate, 5-MTHF, folate, homocysteine, cystathionine, cysteine, and methionine were imported from the PubChem library (https://pubchem.ncbi.nlm.nih.gov/docs/about). 3D protein structures of methionine synthase, cystathionine ß-synthase, and MTHFR were retrieved from the RCSB Protein Data Bank (RCSB PDB, https://www.rcsb.org and then imported into UCSF Chimera-X (https://www.cgl.ucsf.edu/chimerax/). The molecules are displayed with molecular surfaces colored by amino acid hydrophobicity.

Results

In Study 1, 85 patients who visited the clinic with complaints of memory impairment from May 2018 to February 2023 were enrolled. In Study 1, the patients who had been prescribed any supplement containing vitamin B_{12} or folate prior to visiting the clinic were excluded. Background profiles of the subjects in Study 1 are shown in Table 1. They were aged 69.4 years on average with no sex difference. Sex differences were detected in body height, body weight, BMI, creatinine, plasma Hcy, and MMSE score. The average plasma Hcy level in men (11.9 ± 5.3 nmol/mL) was significantly higher than that in women (9.2 ± 2.2 nmol/mL) (p=0.0019). The average MMSE score in men (25.8 ± 5.2) was significantly higher than that in women (22.3 ± 7.9) (p=0.0284). There was no sex difference in serum albumin, MCV, eGFR, TGs, LDL, serum folate, serum vitamin B_{12}, or HbA1c. Their serum concentrations of albumin, eGFR, triglyceride (TG), LDL, and HbA1c were within the reference range, suggesting that these subjects were not generally malnourished.

Table 1: Background profiles of the subjects (Study 1)

	Total (n = 85)	Men (n=34)	Women (n =51)	*p* value
Age	69.4 ± 12.7	68.1 ± 12.4	70.2 ± 12.9	0.4450
Body Hight (cm)	158.2 ± 10.3	166.7 ± 8.4	151.7 ± 6.3	<0.0001**
Body Weight (Kg)	53.5 ± 10.8	62.4 ± 8.9	47.6 ± 7.3	<0.0001**
BMI (Kg/m^2)	21.4 ± 3.1	22.5 ± 3.0	20.7 ± 3.0	0.0114* *
Serum Albumin (g/dL)	4.20 ± 0.29	4.20 ± 0.31	4.20 ± 0.29	0.9881
MCV (fL)	94.6 ± 4.8	94.4 ± 3.8	94.8 ± 5.4	0.6538
Creatinine (mg/dL)	0.72 ± 0.30	0.88 ± 0.43	0.62 ± 0.11	0.0001**
eGFR (mL/ min/1.73m^2)	73.9 ± 15.3	73.9 ± 17.6	73.8 ± 13.7	0.9714
TG (mg/dL)	103.6 ± 50.6	112.4 ± 44.6	97.7 ± 53.8	0.1901
LDL (mg/dL)	124.2 ±37.6	125.3 ± 42.3	123.4 ± 34.5	0.8154
Plasma Hcy (nmol/ mL)	10.3 ± 3.9	11.9 ± 5.3	9.2 ± 2.2	0.0019**
Serum Folate (ng/mL)	16.1 ± 14.6	15.1 ± 11.3	16.8 ± 16.5	0.6020
Serum Vitamin B_{12} (pg/mL)	590.8 ± 451.8	526.4 ± 358.4	633.7 ± 503.4	0.2863
HbA1c (%)	5.45 ± 0.31	5.46 ± 0.30	5.44 ± 0.31	0.6899
CRP (mg/dL)	0.078 ±0.145	0.112 ± 0.184	0.056 ± 0.107	0.0826
MMSE	23.7 ± 7.1	25.8 ± 5.2	22.3 ± 7.9	0.0284*

Data are expressed as mean ± SD.
Comparison was made according to gender using Student t test.
**Statistically significant (p<0.05).*
***Statistically significant (p<0.01).*

Plasma Hcy concentrations were higher in the MCI and dementia groups than in the normal cognitive function group.

Then, the subjects in Study 1 were categorized into normal cognitive function (n = 43), MCI (mild cognitive impairment) (n = 18), and dementia (n = 24) groups according to MMSE score, as mentioned in the Methods section (Table 2). To analyze the association between cognitive function and biomarkers such as body height, body weight, BMI, serum albumin, MCV, creatinine, eGFR, TGs, LDL, plasma Hcy, serum folate, serum vitamin B_{12}, HbA1c, and CRP, the values of these biomarkers among the normal control group, MCI group, and dementia group were compared using ANOVA (Table 2). There were significant differences in age and body height between the MCI and normal control groups and between the normal dementia and control groups. A significant difference was also found in HbA1c between the MCI and normal control groups (P=0.0377). Although not statistically significant, the plasma Hcy concentration was found to be higher in the MCI group (10.79 ± 2.49 nmol/mL) and dementia group (11.00 ± 4.90 nmol/mL) than in the normal cognitive function group (9.74 ± 3.84 nmol/mL). The serum concentration of folate was found to be lower in the MCI group (15.1 ± 10.03 ng/mL) and dementia group (14.5 ± 15.7 ng/mL) than that in the normal cognitive function group (17.5 ± 15.6 ng/mL). There was no difference in BMI, serum albumin, MCV, creatinine, eGFR, TG, LDL, serum vitamin B_{12}, or CRP among the normal control, MCI and dementia groups (Table 2).

Table 2: Characteristics of the subjects according to MMSE score (Study 1).

	Normal (n = 43)	MCI (n =18)	Dementia (n =24)	*p* value
Age	63.6 ±11.9	74.3 ± 11.2**	76.1 ± 10.2**	<0.0001**
Body Hight (cm)	161.6 ± 10.4	155.2 ± 10.3**	152.7 ± 7.3**	0.0012**
Body Weight (Kg)	54.7 ± 12.0	54.7 ± 9.2	50.5 ± 9.2	0.2731
BMI (Kg/m^2)	20.8 ± 3.1	22.6 ± 2.4	21.6 ± 3.4	0.1027
Serum Albumin (g/dL)	4.25 ± 0.27	4.13 ± 0.34	4.17 ± 0.29	0.2993
MCV (fL)	94.2 ± 5.0	95.7 ± 4.8	94.8 ± 4.5	0.5313
Creatinine (mg/dL)	0.70 ± 0.16	0.71 ± 0.15	0.76 ± 0.53	0.7541
eGFR (mL/ min/1,73m^2)	77.1 ± 13.8	70.7 ± 12.7	70.5 ± 18.7	0.1496
TG (mg/dL)	98.0 ± 52.3	119.8 ± 60.1	101.3 ± 37.5	0.3033
LDL (mg/dL)	122.0 ±34.4	135.8 ± 42.4	119.4 ± 39.2	0.3294
Plasma Hcy (nmol/ mL)	9.74 ± 3.84	10.79 ± 2.49	11.00 ± 4.90	0.3931
Serum Folate (ng/mL)	17.5 ± 15.6	15.1 ± 10.03	14.5 ± 15.7	0.6827
Serum Vitamin B_{12} (pg/mL)	652.8 ± 509.6	434.4 ± 253.5	596.9 ± 444.1	0.2284
HbA1c (%)	5.38 ± 0.25	5.59 ± 0.33*	5.47 ± 0.35	0.0377*
CRP (mg/dL)	0.06 ± 0.11	0.14 ± 0.25	0.07 ± 0.08	0.1658
MMSE	28.9 ± 1.1	24.2 ± 1.4**	14.0 ± 5.6**	<0.0001**

Data are expressed as mean ± SD.
Comparison was carried out using ANOVA, followed by Tukey-Kramer muyltiple comparison test.
**Statistically significant (p<0.05).*
***Statistically significant (p<0.01).*

Correlation of plasma Hcy, serum folate and vitamin B_{12} concentrations

To elucidate the association of the biomarkers listed in Study 1 with plasma Hcy, two-way ANOVA showed that plasma Hcy was significantly correlated with serum folate, serum vitamin B_{12}, creatinine and eGFR. As shown in Figure 1A, serum folate was negatively correlated with plasma Hcy (r = -0.297, p=0.0088) with the linear regression equation "Folate = 29.979058–1.3541775*Hcy". Serum vitamin B_{12} was negatively correlated with plasma Hcy (r = -0.297, p=0.0140) with the linear regression equation "VB12 = 971.27356–37.975914*Hcy" (Figure 1B). Serum creatinine was positively correlated with plasma Hcy (r = 0.498, p<0.001) with the linear regression equation "Cre

= 0.4704215 + 0.0227039*Hcy" (Figure 1C). Finally, eGFR was negatively correlated with plasma Hcy (r = -0.280, p=0.0137) with the linear regression equation "eGFR = 86.350383-1.2094062*Hcy" (Figure 1D).

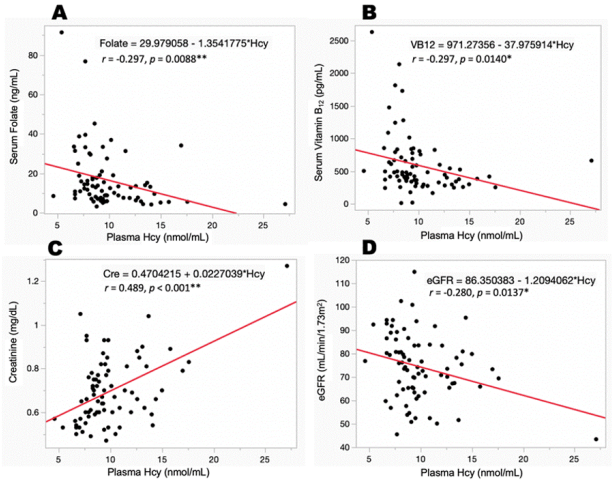

Figure 1: Correlation of Hcy with folate, vitamin B_{12}, creatinine, and eGFR. Correlation between Hcy and serum folate (A), serum vitamin B_{12} (B), serum creatinine (C), and eGFR (D). The linear regression line is shown by the red line. The linear regression equation, r value and p value are shown in the upper panel of the graph. The r value represents Spearman's correlation coefficient. *Statistically significant (p<0.05). **Statistically significant (p<0.01).

Administration of Memorin® lowered the plasma Hcy concentration in HHcy patients

In Study 2, Memorin® was prescribed for 10 months to 42 patients from Study 1 who showed HHcy in the first visit. Memorin® is a dietary supplement containing curcuminoids, DHA, squalene, turmeric powder, piperine, vitamin B_2, vitamin B_6, vitamin B_{12}, folic acid, vitamin C, vitamin E, vitamin B_1 and huperzine A. Plasma Hcy, LDL, TG, vitamin B_{12}, folate, and MMSE were assessed 4 months and 10 months after Memorin® administration. The initial average plasma Hcy concentration in the Study 2 group (12.3 ± 4.58 nmol/mL) significantly decreased after 4 months (10.0 ± 3.85 nmol/mL) and 10 months (10.16 ± 3.12 nmol/mL) of Memorin® administration (p<0.01) (Table 3, Figure 2A). The initial average serum vitamin B_{12} concentration (726.6 ± 1717.2 pg/mL) significantly increased after 4 months (1018.1 ± 1829.6 pg/mL) and 10 months (1042.9 ± 1568.8 pg/mL) of Memorin® administration (p< 0.01) (Table 3, Figure 2D). The initial average serum folate concentration (19.4 ± 33.7 ng/mL) significantly increased after 4 months (43.0 ± 86.3 ng/mL) and after 10 months (32.5 ± 16.9 ng/mL) of Memorin® administration (p< 0.01) (Table 3, Figure 2E). However, no significant changes were found in LDL, TG, and MMSE scores before and 4 months or 10 months after administration of Memorin® (Table 3, Figure 2).

Table 3: Biomarkers before and after the administration of Memorin (Study 2, n = 42)

Biomarkers	Before	4 month	10 month
Hcy (nmol/mL)	12.3 ± 4.58	10.0 ± 3.85**	10.16 ± 3.12**
LDL (mg/dL)	122.0 ± 36.3	127.6 ± 32.2	125.2 ± 31.2
TG (mg/dL)	103.2 ± 49.0	118.1 ± 85.8	116.3 ± 76.1
Vitamin B_{12} (pg/mL)	725.6± 1717.2	1018.1 ± 1829.6**	1042.9 ± 1568.8**
Folate (ng/mL)	19.4 ± 33.7	43.0 ± 86.3**	32.5 ± 16.9**
MMSE score	22.8 ± 7.3	22.3 ± 7.9	21.8 ± 8.7

Data are expressed as mean ± SD.
Comparison was carried out using repeated measures ANOVA.
***Statistically significant (p<0.001).*

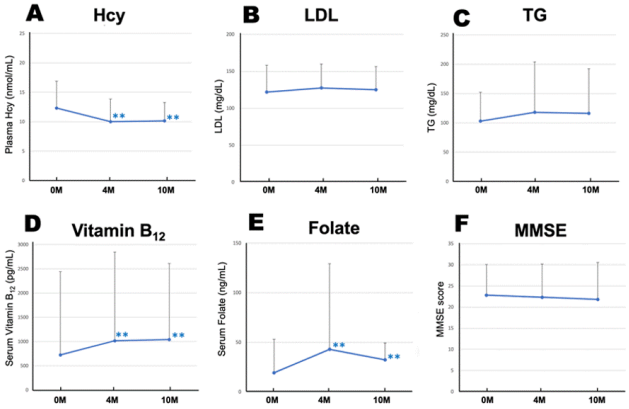

Figure 2: Plasma Hcy, LDL, triglyceride (TG), vitamin B_{12}, serum folate, and MMSE scores of 42 patients before, 4 months, and 10 months after the administration of Memorin®. Data are expressed as the mean + SD. Comparisons were carried out using repeated-measures ANOVA. ** Statistically significant (p<0.01).

Discussion

In the present study, a case–control design was applied to examine the associations of plasma Hcy, serum vitamin B_{12}, folate and cognitive function in patients with normal cognitive function, MCI, and dementia. The plasma Hcy concentration was higher in MCI and dementia patients than in the normal cognitive function group. The pharmacological effect of Memorin®, a commercially available vitamin-B complex dietary supplement, was evaluated on the lowering effect of plasma Hcy in patients with HHcy.

There have been reports on the association between folate, vitamin B_{12} or Hcy and cognitive function [8,10,16,17]. HHcy has been reported to be an independent risk factor for cognitive decline in a case–control study of MCI and AD in China [8] and an Italian cohort study on dementia and

AD [10]. In the current study, the plasma Hcy concentration was elevated in the MCI and dementia groups compared with those in patients with normal cognitive function, but the differences were not statistically significant. One of the factors that limited the statistical significance may be the smaller number of patients with HHcy enrolled in the current study compared to the previously published reports [8,10]. A larger number of cases and controls would be needed to achieve statistical significance. In the current study, a sex difference was found in plasma Hcy, in which men showed significantly higher Hcy concentrations than women (Table 1). A previous study, the cross-sectional Coronary Risk of Insulin Sensitivity in Indian Subjects study (CRISIS), showed a sex difference in Hcy between men and women [18]. The authors suggested that sex differences in plasma Hcy may be related to sex hormone concentrations, lifestyle factors such as diet and smoking, and sexual dimorphism in gene expression that contribute to differences in body composition [18]. Mudd and Poole also suggested that the difference in Hcy between men and women may be related to creatine or creatinine synthesis and the higher muscle mass in men [19]. This possibility is compatible with the positive correlation observed between plasma Hcy and serum creatinine concentration in Study 1 (Figure 1C).

Metabolically, Hcy is either metabolized to methionine by methionine synthase or to cystathionine by cystathionine ß-synthase, as shown in Figure 3. Methionine synthase is one of two enzymes that require cobalamin or vitamin B_{12} for their enzymatic activity and catalyzes the transfer of a methyl group from N^5-methyltetrafolate to homocysteine, generating tetrahydrofolate (THF) and methionine (Figure 3). Vitamin B_{12} serves as an intermediate methyl group carrier and cycles between methylcobalamin and cob(*I*) alamin [20]. Cystathionine ß-synthase catalyzes the first step of the transsulfuration pathway from homocysteine to cystathionine, uses pyridoxal-phosphate (vitamin B_6) as a cofactor, and is allosterically regulated by effectors such as the ubiquitous cofactor S-adenosyl-L-methionine (AdoMet) [21]. Methylenetetrahydrofolate reductase (MTHFR) is the rate-limiting enzyme in the methyl cycle and catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-NTHFR), a cosubstrate for Hcy remethylation to methionine (Figure 3). Some genetic variations in the MTHFR gene have been reported to influence susceptibility to occlusive vascular disease, neural tube defects, Alzheimer's disease and other forms of dementia, colon cancer, and acute leukemia [22].

As a dietary supplement, Memorin® not only contains a vitamin-B complex, including vitamin B_1, B_2, B_6, B_{12}, and folate, but also curcuminoid, turmeric powder, and piperine, which may facilitate the absorption of vitamin B from the gastrointestinal tract. When HHcy is not improved after 4 months of administration of Memorin® for 4 months, an intravenous administration of a vitamin-B complex was applied for HHcy patients who poorly absorb B vitamins from the gastrointestinal route.

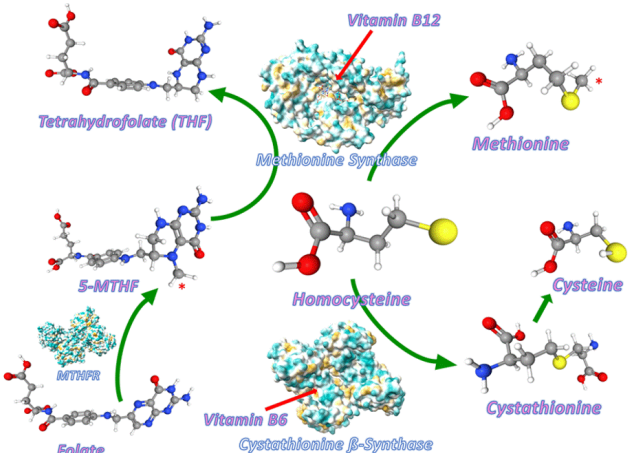

Figure 3: Homocysteine degradation pathway. Homocysteine is catalyzed by methionine synthase to methionine in concomitant catalysis of 5-MTF (5-methyltetrahydrofolate) to THF (tetrahydrofolate). Methionine synthase (PDB ID 1K98) requires vitamin B_{12} as a cofactor. 5-MTHF (serum folate) is catalyzed from folate (dietary origin). Homocysteine is also catalyzed by cystathionine ß-synthase to cystathionine (PDB ID 7XOH) and then further catalyzed to cysteine. Cystathionine ß-synthase requires vitamin B_6 as a cofactor. MTHFR, 5, 10-methyltetrahydrofolate reductase (PDB ID 6FCX). *Methyl residue transferred to methionine by methionine synthase.

No significant improvements were found with MMSE score in Study 2 of the current study, although the majority of patients were administered cytokines that induce neurogenesis and angiogenesis as previously described together with the dietary supplement [23-25]. Various factors, including nutritional status, physical activity levels, and other lifestyle factors, influence the cognitive function of HHcy patients, and further study is needed to define what combination of treatments is optimal for the improvement of cognitive function in HHcy patients.

Conclusion

Higher plasma Hcy concentrations as well as lower serum folate concentrations were associated with cognitive decline in outpatients who visited the clinic. Administration of Memorin® conferred the maintenance of appropriate folate and vitamin B_{12} status and lowered the concentration of plasma Hcy.

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Informed Consent

Written informed consent was obtained from the patients.

Conflict of Interest

The author declares no conflicts of interest.

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