Associations between Alzheimer’s Disease and Blood Homocysteine, Vitamin B₁₂, and Folate: A Case-Control Study

Hui Chen¹, Shuai Liu³, Lu Ji¹, Tianfeng Wu¹, Fei Ma², Yong Ji³*, Yuying Zhou³, Miaoyan Zheng⁴, Meilin Zhang¹ and Guowei Huang¹,*

¹Department of Nutrition and Food Science, School of Public Health, Tianjin Medical University, Tianjin, China; ²Department of Epidemiology and Biostatistics, School of Public Health, Tianjin Medical University, Tianjin, China; ³Department of Neurology, Huanhu Hospital, Tianjin, China; ⁴Metabolic Diseases Hospital & Tianjin Institute of Endocrinology, Tianjin, China

Abstract: Background: There is a growing focus on nutritional therapy for Alzheimer’s disease (AD), and controversy exists regarding the association between AD and homocysteine (Hcy), vitamin B₁₂, and folate levels. Objective: The present study evaluated the association between AD and the combined levels of Hcy, vitamin B₁₂, and folate. Methods: This case-control study consisted of 115 patients with AD and 115 matched controls. Serum folate and vitamin B₁₂ were measured using an automated immunoassay analyzer. Plasma Hcy was measured using high-performance liquid chromatography. The association between AD and Hcy, vitamin B₁₂, and folate was analyzed using binary logistic regression, adjusted for age and sex. Results: With the combination of normal blood Hcy, vitamin B₁₂, and folate levels as the reference category, low vitamin B₁₂ in subjects with normal Hcy and folate was associated with AD (adjusted odds ratio [OR], 4.6; 95% confidence interval [CI]: 1.6–13.2). The combination of low vitamin B₁₂ and folate in subjects with normal Hcy was associated with AD (adjusted OR, 4.3; 95% CI: 1.3–14.6). The combination of high Hcy and low folate levels in patients with normal vitamin B₁₂ was associated with AD (adjusted OR, 17.0; 95% CI: 5.4–53.4). The combination of high Hcy, low vitamin B₁₂, and any folate level was associated with AD (adjusted OR, 30.5; 95% CI: 9.7–95.9). Conclusion: Vitamin B₁₂ was directly associated with AD. The combination of high Hcy, low vitamin B₁₂, and any folate level represented the poorest association with AD.

Keywords: Activities of daily living, Alzheimer’s disease, folate, homocysteine, mini-Mental state examination, vitamin B₁₂.

INTRODUCTION

It is becoming increasingly challenging to control the growing burden of dementia, including Alzheimer’s disease (AD). The World Alzheimer Report 2010 estimated that the ageing of the global population will result in an economic effect of dementia greater than that of cancer, heart disease, and stroke combined [1]. In China, it is estimated that the incidence of AD had reached 6.25 cases per 1000 person-years as of 2010 [1]. Unless effective means to prevent and control this disease are implemented, the economic development of society will likely be hindered.

Homocysteine (Hcy), a thiol-containing amino acid that is generated during 1-carbon metabolism, has been associated with AD in some studies [2–4]. Folate and vitamin B₁₂, both water-soluble vitamins, are coenzymes and cofactors in the methylation of Hcy to methionine, which is a precursor of S-adenosylmethionine. Studies have reported that vitamin B₁₂ and folate levels are lower in AD compared with those in normal controls [2, 5]. Therefore, to improve cognitive function, many clinicians recommend that patients with AD supplement B vitamins to degrade Hcy. However, the combination of low serum vitamin B₁₂ and elevated folate levels is reportedly associated with higher Hcy concentrations and greater odds ratios (ORs) for cognitive impairment than the combination of low vitamin B₁₂ and low or normal folate levels [6].

A number of dietary intervention studies have shown a lack of cognitive improvement despite a successful decrease in Hcy [7, 8] as well as a failure to slow cognitive decline in elderly adults with hyperhomocysteinemia using a B-vitamin supplement [9]. Although it has been reported that Hcy levels are negatively correlated with vitamin B₁₂ and folate levels in vascular dementia, a similar correlation was not found in control subjects or AD patients [10]. Controversy exists over the effect of folate on AD and the relationship of folate with levels of vitamin B₁₂ and Hcy in patients with AD. Recent cross-sectional studies have reinvigorated interest in this issue [6].

The Hcy remethylation pathway using folate and vitamin B₁₂ as a methyl donor and coenzyme, respectively, is just one of the many Hcy metabolic pathways. Therefore, this study aimed to evaluate the associations between AD and blood Hcy, vitamin B₁₂, and folate levels.
MATERIALS AND METHODS

Alzheimer’s Disease Patients and Healthy Volunteers

Between November 2012 and November 2013, 230 elderly patients (115 with AD and 115 controls) were recruited from and diagnosed by neurologists at Neurology Central Hospital of Tianjin. All patients and control subjects were matched 1:1 for age (± 3 years) and sex.

Patients with AD presented with progressive cognitive deficiency for at least 6 months and met the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer’s Disease and Related Disorders Association criteria for probable Alzheimer’s-type dementia [11]. The patients met the following criteria: aged 50–85 years; Hachinski Ischemic Scale score <4; Hamilton Depression Rating Scale score <7; Mini Mental Status Examination (MMSE) [12] score 3.0–24.0 points (mean, 16.6 ± 6.1 points); activities of daily living (ADL) [13] score ≥22.0 (mean, 34.5 ± 11.6 points); cerebral computed tomography or magnetic resonance imaging showing varying degrees of dilatation of the ventricles, medial temporal lobe, and hippocampus with atrophy and widening of the cerebral sulci and other cortical atrophy; and absence of encephalopathy, which has clinical signs similar to AD such as dementia, hydrocephalus, and cerebral infarction, all of which have causes other than AD.

Inclusion criteria for the control group were as follows: no serious physical disease, ability to complete the neuropsychological tests, and absence of any known neurological disorder or cognitive impairment, with an MMSE score >24.0 (mean, 25.5 ± 1.1).

The patients and controls were excluded if they were taking medications that alter folate, B12, or Hcy concentrations. Rather than signifying good vitamin B12 status, very high plasma vitamin B12 concentrations reflect malignant hematopathies or other serious conditions [14, 15]. Consequently, subjects with blood vitamin B12 concentrations >701 pmol/L (950 pg/mL) were excluded [16]. All participants had normocytic anemia (mean corpuscular volume, 82–95 fl) and normal renal function (creatinine clearance, 80–120 mL/min).

Socioeconomic, Clinical, and Anthropometric Assessment

Information on education (years), weight (kg), height (m), right-handedness (yes/no), marital status (married/divorced or widowed), living with others (yes/no), smoking status (yes/no), drinking status (yes/no), comorbid diseases (diabetes, hypertension, hyperlipidemia, or coronary heart disease; yes/no), family history (yes/no), B12 supplement use (yes/no), and folate supplement use (yes/no) was collected using a self-reported questionnaire. Body mass index (BMI) was calculated as weight/height² (kg/m²).

The Chinese government has yet to introduce mandatory fortification of food with folic acid. Given the differences that we detected between the groups in folic acid levels and folic acid supplementation, as described in the Results section, we also chose to report data regarding the use of folic acid and vitamin B12 supplements.

Mini-Mental State Examination and Activities of Daily Living

Cognitive function was assessed using a version of the MMSE, which is a brief, crude, dementia-screening instrument consisting of the following 16 individual questions or simple tasks: naming objects; repeating and remembering a series of three common words; copying a figure; writing a sentence; repeating a phrase; spelling a word backward; and folding a piece of paper and placing it on a desk, table, or floor. Functions assessed include orientation (10 points), registration (3 points), attention and calculation (5 points), recall (3 points), and language and praxis (9 points) [12].

All of the patients were also asked to report whether they had any difficulty with any of the following 20 ADL [17] items: toileting, eating, dressing, grooming, sitting or standing or getting in and out of bed, collecting water for cooking, being at home alone, going out on foot, walking in a flat indoor environment, walking up and down stairs, bathing, cutting toenails, using the telephone, daily shopping, preparing meals, doing housekeeping, doing laundry, taking the bus, taking medicine, and handling personal finances.

Blood Sample Collection

On the morning following hospital admission, blood samples were drawn via vein puncture after at least a 12-h fast; of the two tubes that were collected for each patient, one contained ethylenediaminetetraacetic acid (EDTA) anticoagulant, and the other contained coagulant. The tube containing EDTA anticoagulant was immediately centrifuged at 2,500 g for 10 min at 4°C; then, the plasma samples were obtained and stored frozen at -80°C for subsequent measurement of Hcy. The tube containing coagulant was centrifuged at 3,000 g for 10 min, and the serum samples were obtained. At the same time, a portion of each serum sample was stored frozen at -80°C for the subsequent measurement of serum folate and vitamin B12 levels.

Measurement of Biochemical Parameters

Serum folate and vitamin B12 levels were measured using an automated immunoassay analyzer (Abbott AxSYM system, Abbott Laboratories, Abbott Park, IL, USA) [18, 19]. In this assay, folate was quantified by measuring the population of unoccupied folate binding protein sites bound to the matrix using a conjugate of pteroic acid (folate analog) and alkaline phosphatase as the signal-generating molecule and the substrate 4-methylumbelliferyl phosphate. Similar to folate, the serum level of vitamin B12 was measured using the Abbott kit based on a microparticle enzyme immunoassay. Plasma levels of Hcy were analyzed with high-performance liquid chromatography (HPLC) as described by Poirier et al. [20], with slight modifications. Separation of plasma Hcy was accomplished using HPLC with a 700 HPLC Pump (Waters, Milford, MA, USA) and a reversed-phase C18 column (5-μm bead size; 4.6 × 250 mm) (Waters, Milford, MA, USA). The mobile phase consisted of 0.08 M acetate buffer and 5% (v/v) methanol adjusted to pH 4.0 by addition of concentrated acetic acid and then filtered through a 0.45-μm membrane filter. The isocratic elution was performed using a flow rate of 1.0 mL/min at 30°C and a pressure of 100–110
kgf/cm² (1,500–1,800 psi). A fluorescence detector with excitation at 390 nm and emission at 470 nm was used to detect Hcy. Before analysis of Hcy, the system was calibrated with authentic DL-Hcy standards in the range of 50–4,000 ng. Plasma Hcy was quantified relative to the standard obtained from Sigma Chemical Co. (St. Louis, MO, USA). The inter-assay coefficients of variation for folate were 3.9% and 1.7% at the levels of 3.6 ng/mL and 10.8 ng/mL, respectively, and for vitamin B₁₂ were 4.8% and 3.4% at the levels of 262.0 pg/mL and 424.2 ng/mL, respectively. The relative standard deviations of plasma Hcy were 7.2% and 5.3% at the level of 8.3 μmol/L and 14.2 μmol/L, respectively.

Serum folate concentrations <3.0 ng/mL are traditionally considered to indicate folate deficiency [21]. However, the greatest reduction in neural tube defects has been observed at serum concentrations >7.0 ng/mL [22, 23]. According to the instructions for the Abbott AxSYM system, the reference range for serum folate is 7.0–31.4 ng/mL, suggesting that the desirable range for blood folate in the elderly may need to change to reduce the incidence of neurodegeneration. Various studies have set the normal and low reference values for folate at >7.2 and ≤7.2 ng/mL, respectively [24]. Thus, we classified serum folate levels as low or normal using a cut-off value of 7.0 ng/mL.

Diseases of the nervous system rarely occur owing to a lack of vitamin B₁₂; particularly with B₁₂ levels 300.0–400.0 pg/mL; therefore, we used a cut-off value of 400.0 pg/mL to delineate normal vitamin B₁₂ levels from low vitamin B₁₂ levels [25, 26]. Normal and high Hcy levels were set at <15.0 and ≥15.0 μmol/L, respectively [27-29].

**Statistical Analysis**

All statistical tests were performed using SPSS for Windows v.15.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD), geometric mean ± SD, or proportions. Statistical significance was set at \( P < 0.05 \). Comparisons between categorical variables were conducted using Chi-square tests. Quantitative variables were checked for normality using histograms and quartile-quantile plots. Age, education (years), BMI, MMSE, and ADL were normally distributed and evaluated using two-tailed Student’s \( t \)-tests. Folate, vitamin B₁₂, and Hcy levels, which were normally distributed after logarithmic transformation, were analyzed using analysis of covariance, adjusted for sex and age.

Seven categories of biochemical status were formed: group 1, normal Hcy, vitamin B₁₂, and folate; group 2, normal Hcy and vitamin B₁₂ and low folate; group 3, normal Hcy and folate and low vitamin B₁₂; group 4, normal Hcy and low vitamin B₁₂ and folate; group 5, high Hcy and normal vitamin B₁₂ and folate; group 6, high Hcy, normal vitamin B₁₂ and low folate; group 7, high Hcy, low vitamin B₁₂, and any folate level. Group 7 was formed from 2 other groups because of small cell counts (n=8); the group with high Hcy, low vitamin B₁₂, and normal folate levels was merged with the group with high Hcy, low vitamin B₁₂, and low folate levels.

Differences between AD and control subjects were assessed using binary logistic regression, with diagnosis as the dependent variable and the biochemical status categories as the independent variable. Crude ORs are presented as well as the ORs adjusted for sex and age. The reference category was group 1, followed by groups 2 to 6 in sequence.

**Ethical Considerations**

Each patient or healthy control had a responsible caregiver. All subjects and their caregivers provided written informed consent. The study was approved by the Ethics Committee of the Tianjin Health Service.

**RESULTS**

**Subject Characteristics and Study Outcomes**

Of the 230 individuals (88 men and 142 women) that agreed to participate, patients with AD accounted for 115 participants (44 men and 71 women; age, 53–84 years), and healthy elderly controls also accounted for 115 participants (44 men and 71 women; age, 54–86 years) (Table 1).

There were no significant differences between the two groups in education (\( P = 0.407 \)), handedness (\( P = 1.000 \)), living with others (\( P = 0.116 \)), B₁₂ supplement use (\( P = 0.662 \)), and folate supplement use (\( P = 1.000 \)); however, marital status (\( P = 0.005 \)), BMI (\( P = 0.000 \)), smoking (\( P = 0.004 \)), alcohol use (\( P = 0.002 \)), comorbid diseases (\( P = 0.001 \)), family history (\( P = 0.000 \)), MMSE score (\( P = 0.000 \)), and ADL score (\( P = 0.000 \)) were different between the two groups. In the AD group, plasma Hcy levels were higher (\( P = 0.000 \)), while serum vitamin B₁₂ (\( P = 0.000 \)) and serum folate (\( P = 0.000 \)) levels were lower than in the control group (Table 1).

The ranges of blood folate, vitamin B₁₂, and Hcy levels across the biochemical status categories were 1.0–23.1 ng/mL, 135.1–950.3 pg/mL, and 3.3–37.4 μmol/L, respectively (Table 2).

**Association Between Alzheimer’s Disease and Combined Low Vitamin B₁₂, Low Folate, and High Hcy Levels**

With group 1 as the reference category, the age- and sex-adjusted OR values were 2.2 (95% CI: 0.9–5.5) for those with normal Hcy and vitamin B₁₂ and low folate (group 2), 4.6 (95% CI: 1.6–13.2) for those with normal Hcy and folate and low vitamin B₁₂ (group 3), 4.3 (95% CI: 1.3–14.6) for those with normal Hcy and low vitamin B₁₂ and folate (group 4), 6.4 (95% CI: 1.9–21.6) for those with high Hcy and normal vitamin B₁₂ and folate (group 5), 17.0 (95% CI: 5.4–53.4) for those with high Hcy, normal vitamin B₁₂, and low folate (group 6), and 30.5 (95% CI: 9.7–95.9) for those with high Hcy, low vitamin B₁₂, and normal or low folate levels (group 7) (Table 3).

There was a greater association between AD and the combination of high Hcy, normal vitamin B₁₂, and low folate levels (group 6) (OR and adjusted OR >1) compared with the combination of normal Hcy and vitamin B₁₂ and low folate levels (group 2) or normal Hcy and folate and low vitamin B₁₂ levels (group 3) (Table 3). There was also a greater association between AD and the combination of high Hcy, low vitamin B₁₂, and any folate levels (group 7) (OR and adjusted OR >1) compared with the combination of normal...
Table 1. Baseline characteristics of the study participants.

<table>
<thead>
<tr>
<th>Index</th>
<th>AD (n = 115)</th>
<th>NE (n = 115)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (mean ± SD)</td>
<td>67.6 ± 7.9</td>
<td>66.7 ± 6.2</td>
<td>0.359</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>44 (38.3)</td>
<td>44 (38.3)</td>
<td>1.000</td>
</tr>
<tr>
<td>Education (years) (mean ± SD)</td>
<td>9.1 ± 4.1</td>
<td>9.4 ± 2.4</td>
<td>0.407</td>
</tr>
<tr>
<td>Right-handedness, n (%)</td>
<td>109 (94.8)</td>
<td>109 (94.8)</td>
<td>1.000</td>
</tr>
<tr>
<td>Marital status (married), n (%)</td>
<td>91 (79.1)</td>
<td>106 (92.2)</td>
<td>0.005**</td>
</tr>
<tr>
<td>Living with others, n (%)</td>
<td>105 (91.3)</td>
<td>110 (95.7)</td>
<td>0.116</td>
</tr>
<tr>
<td>BMI (kg/m²) (mean ± SD)</td>
<td>23.6 ± 3.9</td>
<td>25.6 ± 3.3</td>
<td>0.000**</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>26 (22.6)</td>
<td>10 (8.7)</td>
<td>0.004**</td>
</tr>
<tr>
<td>Alcohol use, n (%)</td>
<td>23 (20.0)</td>
<td>7 (6.1)</td>
<td>0.002**</td>
</tr>
<tr>
<td>Comorbid diseases, n (%)</td>
<td>63 (54.8)</td>
<td>37 (32.2)</td>
<td>0.001**</td>
</tr>
<tr>
<td>Family history, n (%)</td>
<td>33 (28.7)</td>
<td>0 (0.0)</td>
<td>0.000**</td>
</tr>
<tr>
<td>B₁₂ supplement use, n (%)</td>
<td>3 (2.6)</td>
<td>1 (0.9)</td>
<td>0.622</td>
</tr>
<tr>
<td>Folate supplement use, n (%)</td>
<td>3 (2.6)</td>
<td>2 (1.7)</td>
<td>1.000</td>
</tr>
<tr>
<td>MMSE (mean ± SD)</td>
<td>16.6 ± 6.1</td>
<td>25.5 ± 1.1</td>
<td>0.000**</td>
</tr>
<tr>
<td>ADL (mean ± SD)</td>
<td>34.5 ± 11.6</td>
<td>20.4 ± 1.9</td>
<td>0.000**</td>
</tr>
<tr>
<td>Hcy (μmol/L) (geometric mean ± SD)</td>
<td>15.1 ± 6.8</td>
<td>9.9 ± 3.8</td>
<td>0.000**</td>
</tr>
<tr>
<td>Vitamin B₁₂ (pg/mL) (geometric mean ± SD)</td>
<td>408.7 ± 192.9</td>
<td>566.0 ± 241.0</td>
<td>0.000**</td>
</tr>
<tr>
<td>Folate (ng/mL) (geometric mean ± SD)</td>
<td>5.4 ± 3.6</td>
<td>7.6 ± 3.3</td>
<td>0.000**</td>
</tr>
</tbody>
</table>

AD, Alzheimer’s disease; NE, normal elderly; BMI, body mass index; MMSE, Mini-Mental State Examination; ADL, activities of daily living; Hcy, homocysteine; n, number of individuals

Comorbid conditions include diabetes, hypertension, hyperlipidemia, and coronary heart disease. Age, education, BMI, MMSE, and ADL, which were normally distributed, were compared using two-tailed Student’s t-tests. Folate, vitamin B₁₂, and Hcy, which were normally distributed after logarithmic transformation, were analyzed by analysis of covariance, adjusted for sex and age. Chi-squared tests and Fisher’s exact tests were performed for frequencies analyses.

**P < 0.01

Table 2. Categories based on the combination of blood folate, vitamin B₁₂, and homocysteine Levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hcy</th>
<th>Vitamin B₁₂</th>
<th>Folate</th>
<th>n</th>
<th>Hcy 3.31–37.4 μmol/L</th>
<th>Vitamin B₁₂ 135.1–950.3 pg/mL</th>
<th>Folate 1.04–23.1 ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>68</td>
<td>8.3 ± 2.5</td>
<td>694.9 ± 223.1</td>
<td>10.0 ± 3.3</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>Normal</td>
<td>Low</td>
<td>41</td>
<td>10.8 ± 2.4</td>
<td>561.9 ± 155.6</td>
<td>4.9 ± 1.2</td>
</tr>
<tr>
<td>3</td>
<td>Normal</td>
<td>Low</td>
<td>Normal</td>
<td>22</td>
<td>8.6 ± 2.4</td>
<td>288.0 ± 82.1</td>
<td>9.6 ± 2.7</td>
</tr>
<tr>
<td>4</td>
<td>Normal</td>
<td>Low</td>
<td>Low</td>
<td>15</td>
<td>11.2 ± 2.4</td>
<td>309.7 ± 70.1</td>
<td>5.2 ± 1.5</td>
</tr>
<tr>
<td>5</td>
<td>High</td>
<td>Normal</td>
<td>Normal</td>
<td>15</td>
<td>20.0 ± 5.9</td>
<td>604.4 ± 175.0</td>
<td>9.1 ± 1.4</td>
</tr>
<tr>
<td>6</td>
<td>High</td>
<td>Normal</td>
<td>Low</td>
<td>28</td>
<td>20.0 ± 5.7</td>
<td>542.4 ± 128.0</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>7</td>
<td>High</td>
<td>Low</td>
<td>Normal/Low</td>
<td>41</td>
<td>19.4 ± 3.8</td>
<td>293.0 ± 69.7</td>
<td>4.1 ± 2.4</td>
</tr>
</tbody>
</table>

Hcy, homocysteine
Due to the small cell counts in the group with high Hcy, low vitamin B₁₂, and normal folate, the group was merged with the group with high Hcy, low vitamin B₁₂, and low folate to form group 7, with high Hcy, low vitamin B₁₂, and low or normal folate levels. The blood Hcy, vitamin B₁₂, and folate cut-off levels were 15.0 μmol/L, 400.0 pg/mL, and 7.0 ng/mL, respectively.

Values are reported as geometric mean ± standard deviation.
Hcy and vitamin B$_{12}$ and low folate levels (group 2), normal Hcy and folate and low vitamin B$_{12}$ levels (group 3), normal Hcy and low vitamin B$_{12}$ and folate levels (group 4), or high Hcy and normal vitamin B$_{12}$ and folate levels (group 5).

**DISCUSSION**

With group 1 as the reference category, the age- and sex-adjusted OR value was 2.2 (95% CI: 0.9–5.5) for those with normal Hcy and vitamin B$_{12}$ and low folate. The evaluation of the associations between AD and combined high Hcy, low vitamin B$_{12}$, and low folate levels in subjects with normal Hcy and vitamin B$_{12}$ (group 2). This finding is somewhat consistent with that of a study in which folate was not related to AD risk [30]. However, the results of another study indicated that low folate levels increase the risk of AD [31], and blood folate levels were significantly lower in AD patients compared with those in normal controls in other studies [5, 32].

With group 1 as the reference category, the age- and sex-adjusted OR value was 4.6 (95% CI: 1.6–13.2) for those with normal Hcy and folate and low vitamin B$_{12}$ (group 3). The findings of the present study also indicated a direct association between AD and low vitamin B$_{12}$ levels in subjects with normal Hcy and folate levels (group 3). A number of studies have suggested that vitamin B$_{12}$ levels are lower in AD patients compared with healthy subjects [2,5,32,33]. At the same time, several longitudinal studies have also linked elevated Hcy levels to an increased risk of AD, dementia, or cognitive decline [30, 34, 35]. Similarly, direct associations between AD and high Hcy levels were found in the presence of normal vitamin B$_{12}$ levels and either normal or low folate levels (groups 5 and 6, respectively).

With group 1 as the reference category, the age- and sex-adjusted OR value was 4.3 (95% CI: 1.3–14.6) for those with normal Hcy and low vitamin B$_{12}$ and folate (group 4), which was another interesting finding of the present study. A recent meta-analysis of a large number of case-control and cross-sectional studies showed that, in subjects with AD, serum vitamin B$_{12}$ and folate levels were lower compared with normal subjects [32]. Low blood levels of vitamin B$_{12}$ or folate have also been related to the development of AD, dementia, or cognitive impairment and to increased rates of brain atrophy [4, 34].

With group 1 as the reference category, the age- and sex-adjusted OR value was 30.5 (95% CI: 9.7–95.9) for those with high Hcy, low vitamin B$_{12}$, and normal or low folate levels (group 7). There was also a greater association between AD and group 7 (OR and adjusted OR >1) compared with group 2, group 3, group 4, or group 5. The associations between AD and the combination of high Hcy and low vitamin B$_{12}$ levels have received less attention in previous studies. In the present study, the combination of high Hcy and low vitamin B$_{12}$ levels in subjects with normal or low folate levels (group 7) presented the worst association with AD. Holotranscobalamin (holoTC) is the biologically active fraction of vitamin B$_{12}$, and lower holoTC concentrations have been reported in patients with AD in a case-control study [36]. Moreover, higher holoTC values have been independently related with a lower risk of AD [30], and results from the Kungsholmen Project showed that moderate (third quartile) but not high (fourth quartile) holoTC levels were associated with reduced AD risk at follow-up [34]. Luchsinger et al. reported that Hcy with low B$_{12}$ status had the strongest association with the combined incidences of dementia and cognitive impairment without dementia [37].

Although the exact mechanisms behind the observed associations are unknown, certain hypotheses could be considered. The association between folate and AD as well as that between vitamin B$_{12}$ and AD may be primarily mediated by Hcy. Folate and vitamin B$_{12}$ are essential cofactors of 1-carbon metabolism, in which a carbon unit from serine or glycine reacts with tetrahydrofolate (THF) to form methyl-
ene-THF. This may be used for the synthesis of thymidylate, a DNA nucleotide, or for purine synthesis. Therefore, B-vitamin deficiency adversely affects DNA synthesis, which is necessary for cell regeneration [38]. Folate or vitamin B12 deficiency is also related to increased Hcy concentrations [39]. Research by Selhub et al suggested that high Hcy levels were associated with low folate levels in patients who also had low vitamin B12 levels [40]. Several mechanisms have been suggested as possible protagonists in the toxic pathway of Hcy in AD onset: oxidative stress and neurotoxicity, vascular damage, alteration of cholesterol and lipids, alteration of protein function by methylation, and deregulation of gene expression by DNA methylation [39].

This study has certain limitations. Because of the cross-sectional nature of the study, the causality of the associations between AD and Hcy, vitamin B12, and folate levels could not be determined. In addition, the relatively small sample size may have resulted in underestimated associations.

CONCLUSION

This investigation aimed at clarifying long-held but evolving ideas regarding the associations between AD and Hcy, vitamin B12, and folate levels. The results indicated an involvement of both high Hcy and low vitamin B12 levels in the development of AD. We found direct associations between AD and low vitamin B12 levels in subjects with normal Hcy and folate levels in addition to an association between AD and low vitamin B12 and folate levels in subjects with normal Hcy levels. Furthermore, the poorest association was between AD and high Hcy and low vitamin B12 levels in subjects with normal folate levels. Despite the partial dependence on Hcy in the association between AD and low vitamin B12 levels, we believe that compensatory mechanisms might partially explain the lower vitamin B12 levels observed in AD. Further investigations of these relationships and their underlying mechanisms are warranted.

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