Low Serum Vitamin B12 in Alzheimer's Patients as Detected by a Solid Phase Radioimmunoassay

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ABSTRACT

Objective: To determine the relationship between serum levels of vitamin B12 and folate in AD and other types of dementia in Thai patients.

Methods: One hundred and eleven Thai subjects were classified into 3 groups: 32 AD patients, 43 non-AD dementia patients and 36 age-matched controls. Serum concentrations of vitamin B12 and folate were measured using a solid phase radioimmunoassay.

Results: Serum vitamin B12 levels were found to be significantly lower in AD and non-AD dementia patients than in age-matched controls. There is a significant relationship between Mini-Mental State Examination score and vitamin B12 level in AD and non-AD dementia groups. However, there is no significant difference in serum folate in AD and non-AD dementia groups when compared to age-matched controls.

Conclusion: These findings suggest the need for vitamin B12 supplementation in AD and non-AD dementia patients.

Keywords: Alzheimer's disease; folate and vitamin B12

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder of unknown etiology, characterized by irreversible cognitive and physical deterioration.[1] AD is the main cause of dementia in elderly people. The diagnosis of AD has been guided by clinical criteria,[2] but a definitive diagnosis of AD is only made when a post-mortem confirmation is available. There are no definitive diagnostic tests or biological markers of AD. There are evidences that oxidative stress, homocysteine-related vitamins, fats and alcohol have roles in the pathogenesis of AD.[3] In AD brains, the pathological signs are defined by the appearance of senile plaque (extracellular insoluble aggregated protein composed of amyloid β, a 39-43 amino acid peptide) and neurofibrillary tangles (intracellular lesions consisting of paired helical filaments from hyperphosphorylated cytoskeletal protein tau).[4]

Many studies found an association between decreased serum levels of vitamin B12 and folate and cognitive ability in Alzheimer's patients.[5,6] Vitamin B12 and folate are important in a number of metabolic pathways in the central nervous system. It has been suggested that the most common causes of vitamin B12 and folate deficiency are inadequate nutrition and mal-absorption, but lifestyle factors, such as smoking, high alcohol consumption and certain diseases, may also affect the metabolism of these vitamins. In addition, aging of the intestinal mucosa might cause a lower degree of absorption and reabsorption.[7] Deficiencies of vitamin B12 and folate result in a high concentration of homocysteine, which is a precursor of methionine and cysteine.[8,9] Methionine is essential for nucleotide biosynthesis and for genomic and non-genomic methylation.[3,10] Wang et al.[6] found that subjects with low levels of vitamin B12 or folate have twice the risks of developing AD.

Assantachai et al.[11] have compared the levels of vitamin B12 and folate between the elderly with good and poor cognitive ability in Thai rural elderly and showed that none of the studied vitamin levels to be significantly different between the two groups. Recent studies

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TABLE 1. Demographics and mean MMSE scores in AD, non-AD dementia and age-matched controls.

	AD	Non-AD dementia	Age-matched control
Number 32	43	36	
M:F ratio	12:20	15:28	14:22
Mean age (SEM)	72.8 ± 1.8	72.2 ± 1.5	68.2 ± 1.1
Mean MMSE score	12.7 ± 3.4*	13.5 ± 5.9*	28.7 ± 5.5

*$p<0.05$ (ANOVA) compared with control. M, male; F, female

demonstrated that AD is not significantly associated with low serum folate and vitamin B12.[12-13] The aim of this study was to determine whether there is a relationship between serum levels of vitamin B12 and folate in AD and other types of dementia in Thai patients using a solid phase radioimmunoassay.

MATERIALS AND METHODS

1. Subjects

Alzheimer's and non-AD dementia patients were diagnosed according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, DSM-IV by the American Psychiatric Association, 1994,[14] with the added provision that subjects had scores of 24 or less on the Mini-Mental State Examination (MMSE). Written informed consent was obtained from all patients or their primary caregivers. The 111 subjects had not been treated with vitamin B12 and folate supplementation before taking blood samples. They were classified into 3 groups: 32 Alzheimer's patients, 43 non-AD dementia patients and 36 age-matched controls.

2. Blood sample analysis

Blood was drawn within 24 h of initial assessment. Serum concentrations of vitamin B12 and folate were measured using a solid phase radioimmunoassay (Radio-assay kit, Diagnostic Products Co., Ltd.). Vitamin B12 deficiency[6] was defined as having a serum concentration < 150 pmol/l and folate deficiency as a serum concentration < 10 nmol/l. A one-way Analysis of Variance (ANOVA) was performed on each variable and the Fisher's PLSD statistic was employed to compare the mean values of patients and controls. Difference is considered statistically significant at a p value < 0.05.

RESULTS

The characteristics of the study groups are shown in Table 1. Patients in all 3 groups did not differ significantly in age and sex ratio. Mean MMSE scores are significantly different, with AD and non-AD dementia subjects being more impaired than the age-matched controls ($p<0.05$).

Table 2 shows the mean values and standard error means (SEM) of serum vitamin B12 and folic acid in AD, non-AD dementia and age-matched controls. There were significant differences between serum vitamin B12 of AD and age-matched controls ($p<0.01$) and between serum vitamin B12 of non-AD dementia and age-matched controls ($p<0.05$). On the other hand, there are no significant differences in serum folate of AD and non-AD dementia groups when compared with age-matched controls. Table 3 shows the percentage of subjects in the three groups with levels of vitamin B12 and folate below the normal range in the three groups. There are no significant differences among all three groups for low folate level, whereas low levels of serum vitamin B12 were seen in AD and non-AD dementia patients.

TABLE 2. Serum vitamin B12 and folate levels in AD, non-AD dementia and age-matched controls.

Subjects	Vitamin B12 (pmol/l) Mean ± SEM	Folate (nmol/l) Mean ± SEM
AD (n = 32)	449.9 ± 25.4**	12.4 ± 3.5
Non-AD dementia (n = 43)	462.2 ± 20.7*	10.3 ± 1.9
Age-matched control (n = 36)	641.9 ± 17.6	12.7 ± 2.5

* $p<0.05$ and ** $p<0.01$ (ANOVA) compared with control. n, numbers of subjects

TABLE 3. Percentage of subjects with vitamin B12 or folate levels below normal range.

	AD n (%)	Non-AD dementia n (%)	Age-matched control n (%)
Serum vitamin B12	4 (12.5)	4 (9.3)	0 (0)
Serum folate	1 (3.1)	2 (4.7)	1 (2.8)

n, numbers of subjects.

DISCUSSION

We found the level of vitamin B12 in the serum of AD and non-AD dementia patients are significantly lower than age-matched controls. These results support the previous reports of Levitt et al.[5] who indicated that serum vitamin B12 level is lower in patients with dementia of Alzheimer type and patients with histologically confirmed AD than in controls. In addition, Wang et al.[6] found that subjects with a low level of serum vitamin B12 or folate have twice the risk of developing AD. However, retrospective studies failed to reveal significant associations between serum vitamin B12 concentration and dementia or AD.[12,15]

In our study we did not find significant differences in serum folate levels among non-AD dementia, AD patients and controls. Moreover, Haan et al.[16] reported that homocysteine is associated with vitamin B12 but no correlation with folate. Elwood et al.[17] found no association between folate status and mental function in AD patients, but Snowdon et al.[18] demonstrated a significant correlation between relatively low folate concentration and severity of postmortem neocortical atrophy.

It is noteworthy that low levels of vitamin B12 are significantly correlated with scores of severity of cognitive impairment in patients with AD and non-AD dementia. Similarly, previous studies have reported a positive correlation between MMSE scores and vitamin B12 levels in AD.[5,6] These findings may suggest the need for vitamin B12 supplemen-tation in AD and non-AD dementia patients.

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