IJMS
Vol 34, No 3, September 2009

Original Article

Serum Levels of Homocysteine, Vitamin B12, and Folic Acid in Patients with Alzheimer's Disease

Fariba Karimi[1], Afshin Borhani Haghighi[2], Payman Petramfar[2]

Abstract

Background: Alzheimer's disease is the most common form of dementia in the elderly. Serum levels of homocysteine have been related to increased cortical and hippocampal atrophy. We aimed to determine the serum levels of homocysteine, folate, and vitamin B_{12} in patients with Alzheimer's disease.

Methods: Blood levels of homocysteine and its biological determinants, folate, and vitamin B12 were measured in 51 patients who were diagnosed as having Alzheimer's disease according to DSM-IV criteria and compared with the serum levels obtained from 49 control individuals.

Results: The mean serum homocysteine concentration was significantly higher in patients with Alzheimer's disease than the controls (20.4 ± 16.5 µmol/L *v* 14.5 ± 5 µmol/L; P= 0.02). There were no statistically significant differences between the mean serum levels of vitamin B_{12} (P=0.6) and folate (P= 0.3) in the patients and the controls. There was no correlation between age and serum homocysteine concentration in both groups (P= 0.8).

Conclusion: Serum homocysteine concentration was significantly higher in the patients with Alzheimer's disease. This biomarker might be considered as a predictor of cognitive performance.

Iran J Med Sci 2009; 34(3): 181-185.

Keywords ● Alzheimer`s disease ● homocysteine ● vitamin B12 ● folate

[1]Departments of Endocrinology,
[2]Neurology,
Medical School,
Shiraz University of Medical Sciences,
Shiraz, Iran.

Correspondence:
Fariba Karimi MD,
Department of Endocrinology,
Medical School,
Shiraz University of Medical Sciences,
Shiraz, Iran.
Tel/Fax: +98 711 6474316
Email: karimif2002@yahoo.com
Received: 8 September 2008
Revised: 6 June 2009
Accepted: 11 March 2009

Introduction

Alzheimer's disease is a progressive neurologic disorder that results in memory loss, personality changes, global cognitive dysfunction, and functional impairments. Loss of short-term memory is most prominent early. In late stages of the disease, patients are totally dependent upon others for basic activities of daily living such as feeding and toileting.[1,2]

Alzheimer's disease is the most common form of dementia in the elderly, accounting for 60-80% of cases. It is estimated to affect more than four million Americans.[3-6] The cause of neuronal death in Alzheimer's disease in key parts of the brain, such as the hippocampus, is not known.

The amino acid homocysteine may be an independent risk factor for cognitive decline and dementia, however, the evidence is conflicting.[7-13] Homocysteine levels are associated with increased cortical and hippocampal atrophy, indirectly supporting a role in Alzheimer's disease.[14-16] Elevated blood homocysteine and cholesterol levels, hypertension, and insufficient exercise are all being explored as the potential risk factors for Alzheimer's disease.[17,18]

Several investigators have found inverse associations between objective measures of cognitive function and plasma homocysteine concentrations in patients with Alzheimer's disease and other psychogeriatric disorders.[18-22] However, other researchers found no significant association in this regard.[23-27]

Homocysteine is also a sensitive indicator of folate and cobalamin deficiencies. Some other studies have demonstrated inverse associations between Alzheimer's disease and folate and vitamin B_{12} levels.[19,28-32]

It is important to reveal, whether there is a relationship between cognitive function and plasma levels of homocysteine, folate, and vitamin B_{12} because they might be modifiable risk factors for dementia. The aim of this study was to explore this potential relationship.

Patients and Methods

Between October 2006 and November 2007, 51 patients aged 55 years or older, who were diagnosed as having Alzheimer's disease according to DSM-IV criteria, were consecutively referred to the senior investigator in Motahari Clinic affiliated to Shiraz University of Medical Sciences. Liver and renal function tests, serum electrolyte levels, Wright, 2-ME (2- mercaptoethanol), and thyroid function tests were requested for all the patients before enrollment in the study. All the patients underwent a brain imaging study (computed tomography or magnetic resonance imaging).

Inclusion criteria were diagnosis of Alzheimer's disease according to DSM-IV criteria, and age of 50 years old or more.

Exclusion criteria were a history of gastric surgery, neoplasia, hepatorenal disease, malabsorption syndromes, vegetarianism, thyroid disease, or using homocysteine, folate, or cobalamin disruptive medications, and evidence of multi-lacunar infarction, normal pressure hydrocephaly, brain mass, subdural hematoma, or any gross pathological findings in neuroimaging except for cortical and hippocampal atrophy. Forty nine volunteers of similar age, without a diagnosis of Alzheimer's disease or other major neurodegenerative diseases who satisfied our exclusion criteria served as control group.

Venous blood samples were obtained for measurement of plasma homocysteine, folate, and vitamin B_{12} from the participants after overnight fasting. Serum separation and freezing were performed within one hour of venipuncture. Serum total homocysteine levels were measured by homocysteine enzyme immunoassay (EIA) (Axis-shield diagnostic. UK). Plasma levels of vitamin B_{12} and folate were measured by simulTRAC-SNB radioimmunoassay (RIA) (DRG instruments. GmbH. Germany).

This study was approved by the ethical committee of Shiraz University of Medical Sciences and informed consents were obtained from the participants.

Statistical Analysis

In primary analyses on 10 persons (five from each group), and based on difference in the mean levels of the two groups (diff = 5.7) and their shared standard deviation (SD = 10) at the error level of alpha = 0.05 and power=80%, the number of participants in two groups was determined as 49 persons in each of them. The data were analyzed using SPSS software version 9. Mean values for age, body mass index, and blood levels of the variables were compared among the patients and controls using independent *t* test and their correlation by Pearson Chi square and Pearson correlation coefficient tests, and P value < 0.05 was considered as statistically significant.

Results

In this case-control study, 51 patients with the diagnosis of Alzheimer's disease (18 women and 33 men) and 49 control individuals (23 women and 26 men) were enrolled. Overall, the patients were older than controls (P=0.005), however, there were no statistically significant differences regarding the gender distribution (P=0.2) and body mass index (P=0.7) between the two groups.

The mean serum homocysteine concentration was significantly higher in patients compared with the controls (P = 0.02). The mean serum levels of vitamin B_{12} and folate in the patients and controls were not statistically different (P=0.6 and P=0.3 respectively). Demographic characteristics and mean levels of the metabolic parameters are summarized in tables 1 and 2.

Discussion

There is controversy on the relationship between serum homocysteine level and cognitive

Table 1: Demographic characteristics of the participants in both groups

Parameter		Control group (n=49)	Patients group (n=51)	P value
Gender	Men	26	33	0.2
	Women	23	18	
Age (mean ± SD)		68 ± 8	75 ± 16	0.005
BMI (kg/m^2)		24 ± 5	25 ± 16	0.7

BMI: body mass index, SD: standard deviation

Table 2: Serum homocysteine, vitamin B_{12}, and folate levels in controls and patients (mean ± SD)

	Control group	Patients group	P value
Homocysteine (µmol/l)	14.5 ± 5	20.4 ± 16.5	0.02
Vitamin B_{12} (pg/ml)	364 ± 286	390 ± 246	0.6
Folate (ng/ml)	7 ± 3.8	6.4 ± 2.9	0.3

SD: standard deviation, There were no correlations between age, gender, and body mass index and serum homocysteine levels in the patients and control groups.

performance. In the present case-control study, we found statistically significant higher serum levels of homocysteine in patients with Alzheimer's disease compared with controls despite similar concentrations of vitamin B_{12} and folate in the two groups. Moderately elevated levels of homocysteine are common in the population and increase with advanced age.[33,34] Several studies have reported that plasma homocysteine concentration is correlated with development or progression of Alzheimer's disease.[7,18] If elevated plasma level of homocysteine is correlated with cognition in different pathological conditions as well as normal aging, it is worthy to find whether this correlation is disease specific. In present study, we found no correlation between serum homocysteine levels and age or gender in the two groups, although control individuals were younger than the patients.

In accordance with our results, McCaddon and co-workers showed in their case-control study that plasma homocysteine level was significantly higher in patients with Alzheimer and this increased level did not mimic the age-related elevation, suggesting a pathological role for hyperhomocysteinemia in senile Alzheimer type of dementia.[22]

Two studies by Renvall and Joosten reported similar findings.[21,35] Seshadri and colleagues in the cohort of Framingham study reported a two fold increased risk of Alzheimer's disease for individuals in the highest quartile of homocysteine levels even after adjustment for age.[7]

On the contrary, Luchsinger and others observed a modest, insignificant association between the highest homocysteine quartiles and Alzheimer disease and found that the association between homocysteine level and the disease was confounded by age.[27]

Some other studies concluded that elevated plasma homocysteine levels did not seem to be a primary cause of Alzheimer's disease but rather a reflection of concomitant vascular disease in such patients.[24-26,36] Ariogul and co-workers did not show any correlation between plasma homocysteine, vitamin B_{12}, and folate levels and cognitive performance.[23] In addition they did not detect any significant correlation between homocysteine levels and a positive history for coronary artery disease or cerebrovascular events.[23]

On the other hand, Bell and colleagues showed a correlation between homocysteine levels and poor cognition in depressed elderly inpatients without cardiovascular disease.[37]

Although the explanations for these findings are not clear, some possible mechanisms may include the direct neurotoxic effect of homocysteine on neuronal tissues and impaired neurotransmitter synthesis that can lead to neuronal degeneration and white matter atrophy.[22,32,38] Homocysteine may activate the N-methyl-D-aspartate receptor or convert into homocysteic acid and indirectly leads to neuronal death.[37,39]

High plasma homocysteine level is a risk factor for vascular disease, however, the association between homocysteine and Alzheimer's disease has been also observed in patients without evidence of cerebrovascular disease. Therefore other possible mechanisms might be considered as the etiology of Alzheimer's disease.[19,28,29,40,41]

It is well known that subcortical ischemic lesions are prevalent in Alzheimer's disease. And postmortem studies have shown that about 60% of patients with Alzheimer's disease have white matter ischemic lesions, though not severe enough to cause complete infarctions.[26] Clarke and colleagues in their case-control study with longitudinal assessment of dementia and subsequent histopathological confirmation of the types of dementia found that the patients with elevated plasma homocysteine levels at the first visit had more rapid atrophy of the medial temporal lobe during a 3-year follow-up than those with lower homocysteine levels.[18]

Plasma homocysteine concentration is a function of a complex interaction between multiple genetic and environmental factors. Some investigators attributed the difference between

homocysteine levels in patients with Alzheimer's disease and controls to nutritional factors and concluded that lower folate and cobalamin levels were likely to be the primary determinants of elevated homocysteine.[11,18,22,23,29,31,41]

Another point to be mentioned is that clinical deficiencies of B vitamins have been implicated in brain-related disorders including reversible dementia, depression, and electrophysiological dysfunction such as convulsions.[21,22,24,32,33] Ellison and co-workers systematically reviewed the studies on the correlation between the serum cobalamin, folate, and homocysteine levels with cognitive function and concluded that there was an association between plasma homocysteine level and cognitive impairment in the elderly, however, they did not find a correlation between low levels of cobalamin or folate and cognitive decline.[42] The results of the present study showed that plasma vitamin B_{12} and folate levels were not different between patients and control subjects.

Ravalgia and colleagues in their prospective study showed that elevated serum homocysteine and low serum folate concentrations are independent predictors of the development of dementia and Alzheimer's disease, whereas the association was not significant for vitamin B_{12}.[30]

McCaddon and others showed no significant role for cobalamin, folate, and retinol binding protein levels as the indicators of nutritional status but they reported a highly significant difference in homocysteine levels between patients with Alzheimer and control subjects.[22]

The association between low serum folate and vitamin B_{12} levels with Alzheimer's disease might be related to their effects on methylation reactions in the brain or can be mediated by their effects on homocysteine levels.[29]

Conclusion

The present study revealed significantly higher plasma homocysteine concentration in patients with Alzheimer's disease. So this biomarker might be considered as a predictor of cognitive performance.

Further investigations are needed to clarify whether this correlation modifies cognitive decline.

Acknowledgement

The present investigation was supported by a grant (No. 86-3442) from the Vice Chancellor for Research, Shiraz University of Medical Sciences. The authors gratefully acknowledge the staff at Shiraz Endocrine Research Center for their collaboration.

Conflict of Interest: None declared

References

1 Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* 1993; 43: 2412-4.
2 Juva K, Sulkava R, Erkinjuntti T, et al. Staging the severity of dementia: comparison of *clinical (CDR, DSM-III-R), functional (ADL, IADL) and cognitive (MMSE) scales. Acta* Neurol Scand 1994; 90: 293-8.
3 Evans DA. Estimated prevalence of Alzheimer's disease in the United States. *Milbank Q* 1990; 68: 267-89.
4 Bachman DL, Wolf PA, Linn R, et al. Prevalence of dementia and probable senile dementia of the Alzheimer type in the Framingham Study. *Neurology* 1992; 42: 115-9.
5 Evans DA, Funkenstein HH, Albert MS, et al. Prevalence of Alzheimer's disease in a community population of older persons. Higher than previously reported. JAMA 1989; 262: 2551-6.
6 Hebert LE, Scherr PA, Bienias JL, et al. Alzheimer disease in the US population: prevalence estimates using the 2000 census. *Arch Neurol* 2003; 60: 1119-22.
7 Seshadri S, Beiser A, Selhub J, et al. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. *N Engl J Med* 2002; 346: 476-83.
8 Kalmijn S, Launer LJ, Lindemans J, et al. Total homocysteine and cognitive decline in a community-based sample of elderly subjects: the Rotterdam Study. *Am J Epidemiol* 1999; 150: 283-9.
9 Wright CB, Lee HS, Paik MC, et al. Total homocysteine and cognition in a tri-ethnic cohort: the Northern Manhattan Study. *Neurology* 2004; 63: 254-60.
10 Garcia A, Zanibbi K. Homocysteine and cognitive function in elderly people. *CMAJ* 2004; 171: 897-904.
11 Kado DM, Karlamangla AS, Huang MH, et al. Homocysteine versus the vitamins folate, B6, and B12 as predictors of cognitive function and decline in older high-functioning adults: MacArthur Studies of Successful Aging. *Am J Med* 2005; 118: 161-7.
12 Irizarry MC, Gurol ME, Raju S, et al. Association of homocysteine with plasma amyloid beta protein in aging and neurodegenerative disease. *Neurology* 2005; 65: 1402-8.
13 Nurk E, Refsum H, Tell GS, et al. Plasma total homocysteine and memory in the elderly: The Hordaland Homocysteine study. *Ann Neurol* 2005; 58: 847-57.
14 Vermeer SE, van Dijk EJ, Koudstaal PJ, et al. Homocysteine, silent brain infarcts, and white matter lesions: the Rotterdam

Scan study. *Ann Neurol* 2002; 51: 285-9.
15 Vermeer SE, Prins ND, den Heijer T, et al. Silent infarcts and the risk of dementia and cognitive decline. *N Engl J Med* 2003; 348: 1215-22.
16 den Heijer T, Vermeer SE, Clarke R, et al. Homocysteine and brain atrophy on MRI of non-demented elderly. *Brain* 2003; 126: 170-5.
17 Hachinsky V. Preventable senility: a call for action against the vascular dementias. *Lancet* 1992; 340: 645-8.
18 Clarke R, Smith AD, Jobst KA, et al. Folate, vitamin B_{12} and serum total homocysteine levels in confirmed Alzheimer's disease. *Arch Neurol* 1998; 55: 1449-55.
19 Nilsson K, Gustafson L, Fäldt R, et al. Hyperhomocysteinemia: a common finding in a psychogeriatric population. *Eur J Clin Invest* 1996; 26: 853-9.
20 Quadri P, Fragiacomo C, Pezzati R, et al. Homocysteine, folate and vitamin B_{12} in mild cognitive impairment, Alzheimer's disease and vascular dementia. *AM J Clin Nutr* 2004; 80: 114-22.
21 Joosten E, Lesaffre E, Riezler R, et al. Is metabolic evidence for vitamin B_{12} and folate deficiency more frequent in elderly patients with Alzheimer's disease? *J Gerontol A Biol Sci Med Sci* 1997; 52: M76-9.
22 Andrew McCaddon, Gareth Davies, Peter Hudson, et al. Total serum homocysteine in senile dementia of Alzheimer type. *Int J Geriatr Psychiatry* 1998; 13: 235-9.
23 Arioğul S, Cankurtaran M, Dağli N, et al. Vitamin B_{12}, folate, homocysteine and dementia: are they really related? *Arch Gerontol Geriatr* 2005; 40: 139-46.
24 Miller JW, Green R, Mungas DM, et al. Homocysteine, vitamin B_6 and vascular disease in AD patients. *Neurology* 2002; 58: 1471-5.
25 Kalmijn S, Launer LJ, Lindemans J, et al. Total homocysteine and cognitive decline in a community-based sample of elderly subjects: the Rotterdam Study. *Am J Epidemiol* 1999; 150: 283-9.
26 Nilsson K, Gustafson L, Hultberg B. Elevated plasma homocysteine concentration in elderly patients with mental illness is mainly related to the presence of vascular disease and not the diagnosis. *Dement Geriatr Cogn Disord* 2007; 24: 162-8.
27 Luchsinger JA, Tang MX, Shea S, et al. Plasma homocysteine levels and risk of Alzheimer disease. *Neurology* 2004; 62: 1972-6.
28 Boushey CJ, Beresford SA, Omenn GS, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. *JAMA* 1995; 274: 1049-57.
29 Selhub J, Jacques PF, Wilson PW, et al. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA* 1993; 270: 2693-8.
30 Ravaglia G, Forti P, Maioli F, et al. Homocysteine and folate as risk factors for dementia and Alzheimer disease. *Am J Clin Nutr* 2005; 82: 636-43.
31 Oh R, Brown DL.Vitamin B_{12} deficiency. *Am Fam Physician* 2003; 67: 979-86.
32 Riggs KM, Spiro A 3rd, Tucker K, Rush D. Relations of vitamin B-12, vitamin B-6, folate, and homocysteine to cognitive performance in the Normative Aging Study. *Am J Clin Nutr* 1996; 63: 306-14.
33 Duthie SJ, Whalley LJ, Collins AR, et al. Homocysteine, B vitamin status, and cognitive function in the elderly. *Am J Clin Nutr* 2002; 75: 908-18.
34 Ravaglia G, Forti P, Maioli F, et al. Homocysteine and cognitive function in healthy elderly community dwellers in Italy. *Am J Clin Nutr* 2003; 77: 668-73.
35 Renvall MJ, Spindler AA, Ramsdell JW, et al. Nutritional status of free- living Alzheimer's patients. *Am J Med Sci* 1989; 298: 20-7.
36 Nilsson K, Gustafson L, Hultberg B. Plasma homocysteine and vascular disease in psychogeriatric patients. *Dem Geriatr Cogn Disord* 2006; 21: 148-54.
37 Bell IR, Edman JS, Selhub J, et al. Plasma homocysteine in vascular disease and in nonvascular dementia of depressed elderly people. *Acta psychiatry Scand* 1992; 86: 386-90.
38 Elisabet Englund, Arne Brun, Lars Gustafson. A white-matter disease in dementia of Alzheimer's type - clinical and neuropathological correlates. *International Journal of Geriatric Psychiatry* 1989; 4: 87-102.
39 Beal MF, Swartz KJ, Finn SF, et al. Neurochemical characterization of excito toxin lesions in the cerebral cortex. *J Neurosci* 1991; 11: 147-58.
40 Clarke R, Daly L, Robinson K, et al. Hyperhomocystinemia: and independent risk factor for vascular disease. *N Engl J Med* 1991; 324: 1149-55.
41 Homocysteine Lowering Trialists, Collaboration. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *BMJ* 1998; 316: 894-8.
42 Ellison M, Thomas J, Patterson A. A critical evaluation of the relationship between serum vitamin B, folate and total homocysteine with cognitive impairment in the elderly. *J Hum Nutr Diet* 2004; 17: 371-383.