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Homocysteine: A risk factor for Alzheimer's Disease?

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Abstract

An elevated blood plasma level of the sulfur amino acid homocysteine, a condition termed hyperhomocystenemia, has been shown to be an independent risk factor for vascular disease. Studies have associated Alzheimer's disease (AD) and mild cognitive impairment (MCI) with higher levels of plasma homocysteine than controls. Despite these efforts, no studies have yet implicated homocysteine in the cognitive trajectories of this disorder. This study aimed to assess plasma homocysteine levels as a potential predictor of Alzheimer's disease. A total of 103 subjects were tested and divided into three diagnostic groups: No Impairment, MCI, and AD. Plasma homocysteine levels were assessed using high-performance liquid chromatography (HPLC) with postcolumn fluorescence detection. No significant differences were observed across diagnostic groups in either homocysteine or cysteine concentrations. A potential explanation for this could be that increased dietary folate, caused by government-mandated folic acid fortification, has decreased homocysteine levels across the U.S. population. Further research is needed to determine whether homocysteine is still a risk factor for AD in this folic acid-fortified era.

Introduction

Dementia, characterized by a progressive decline in mental function, memory, and intellectual skills, affects approximately 2-4% of individuals over the age of 65. Two of the predominant types of dementia are Alzheimer's disease (AD) and vascular dementia. AD accounts for the majority, about 70%, of all cases of dementia. The most prominent features of Alzheimer's disease include reduced overall brain mass, thin cortical gyri, and enlarged ventricles. On a cellular level, accumulation of senile plaques, amyloid proteins, and neurofibrillary tangles contribute to the pathology of the disorder (Miller, 2000).

Although vascular disease and AD have long been classified as two distinct disorders, emerging evidence suggests that vascular disease may play a role in the progression of AD. Notably, authors of the Nun Study found that individuals with definitive postmortem evidence of AD and brain infarcts have more severe cognitive impairment and higher rates of dementia than did those with evidence only of AD (Snowdon, Greiner, Mortimer, Riley, Greiner, & Markesbery, 1997).

Prevoius studies have demonstrated that an elevated level of the non-protein sulfur amino acid homocysteine in the blood plasma, termed hyperhomocysteinemia, is an independent risk factor for vascular disease (Refsum, Ueland, Nygard, & Vollset, 1998). By a similar logic, hyperhomocysteinemia may also contribute to the onset of AD. In fact, studies have shown that patients with clinically confirmed AD or mild cognitive impairment (MCI) have higher levels of blood plasma homocysteine and lower levels of folate than controls (Clarke et al., 1998; Miller et al., 2003; Ramos et al., 2005; Haan et al., 2007).

Hyperhomocysteinemia's role on AD pathology has not yet been elucidated, but there have been several purported mechanisms implicating homocysteine. First, as the

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molecule is a risk factor for vascular disease, elevated levels of plasma homocysteine can lead to changes in vasculature and impair blood coagulation pathways, which then may result in the decreased supply of blood to neurons, causing neuronal death. This hypothesis may explain the deficit of cholinergic neurons that is often observed in patients with AD (Mizrahi, 2002). Second, homocysteine has been shown to have neurotoxic effects *in vitro.* It binds to NMDA receptors in the brain, causing calcium influx and triggering generation of reactive oxygen species (ROS), ultimately fostering apoptosis (Mizrahi, 2002; Morris, 2003). Third, homocysteine may induce DNA breakage in neurons by impaired methylation reactions (Shea, 2002). Lastly, perhaps most trivially, homocysteine simply might not be a causative factor and instead a consequence of the poor dieting habits of those with AD.

Most of the aforementioned studies that investigated the role of homocysteine in AD and vascular disease were cross-sectional in design, making difficult the determination of whether hyperhomocysteinemia is a causative factor of AD or simply a ramification of the disorder. The longitudinal study of cognitive trajectories in older persons conducted by Mungas et al. (2010) showed that such trajectories appear heterogeneous after follow-ups. More recent studies, however, suggested that characteristics such as levels of C-reactive protein and MRI-based brain measurements were closely related to rate of cognitive decline in older patients (Bettcher et al., 2012; Carmichael et al., 2012). Thus, the possibility that plasma homocysteine levels can predict cognitive decline and onset of Alzheimer's disease remains open.

The purpose of this study was to determine whether plasma levels of homocysteine could serve as a predictive biomarker for cognitive decline in older individuals. It was hypothesized that higher levels of plasma homocysteine would predict increased risk of cognitive decline and AD onset.

Methods

Subject information and recruitment

A total of 103 subjects were recruited for this study. Informed consent for this study was obtained from all subjects prior to experimentation. Based on clinical observations, subjects were placed into one of three groups: No Impairment (n=63), Mild Cognitive Impairment (MCI, n=29), and Alzheimer's (AD, n=11). Subjects were relatively age-matched across diagnostic groups, with the mean ages ranging from 73.1 – 77.5 (Table 1).

Table 1. Subject information.

Diagnosis	Males	Females	Mean Age	S.D
No Imp.	22	41	73.1	6.4
MCI	13	16	76.2	5.8
AD	1	10	77.5	6.5

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Assessing plasma homocysteine

Blood samples were collected from patients by standard venipuncture. Plasma cysteine and homocysteine concentrations were measured by high performance liquid chromatography with postcolumn fluorescence detection, which has been shown to be a reliable and rapid way to quantify homocysteine concentrations (Vester & Rasmussen, 1991; Ueland, Refsum, Stabler, Malinow, Andersson, & Allen, 1993). Three quality controls were included in each run to ensure accuracy and reliability of results.

Results

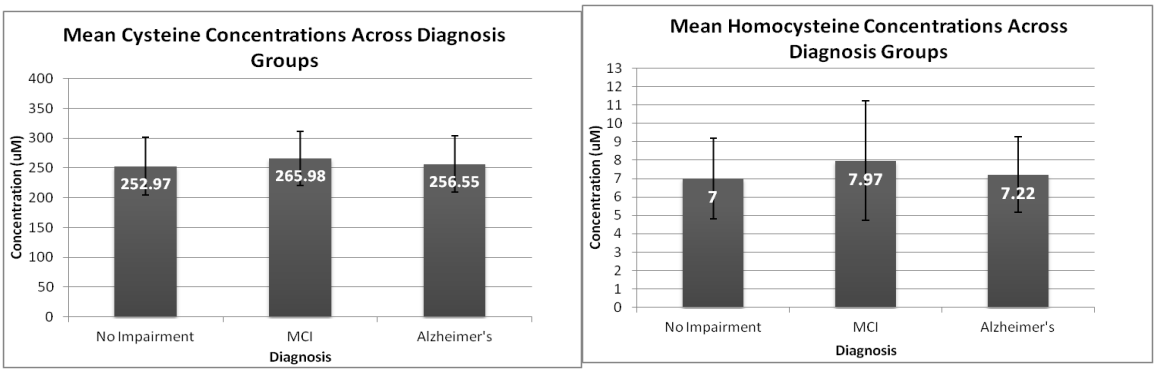

Figure 1a (left) and 1b (right). Mean cysteine and homocysteine concentrations in µmol/L across three diagnostic groups. Error bars represent ±1 S.D. from the mean. No significant difference in cysteine and homocysteine concentrations was observed across all three diagnostic groups.

Data were obtained from all 103 patients. All cysteine and homocysteine concentrations are reported in µmol/L. Figure 1a and Figure 1b show the mean cysteine and homocysteine concentrations across each diagnostic group, respectively. All three diagnostic groups did not significantly differ in their cysteine and homocysteine concentrations.

Discussion

This study found no significant differences in homocysteine and cysteine levels across diagnostic groups, which seems to contradict previous findings that patients with cognitive impairment exhibited higher levels of plasma homocysteine than controls (Clarke et al., 1998; Miller et al., 2003; Ramos et al., 2005; Haan et al., 2007). One plausible explanation involves government-mandated folic acid fortification beginning in 1996. The subjects examined in this study theoretically all had at least several years to experience the effects of folic acid fortification.

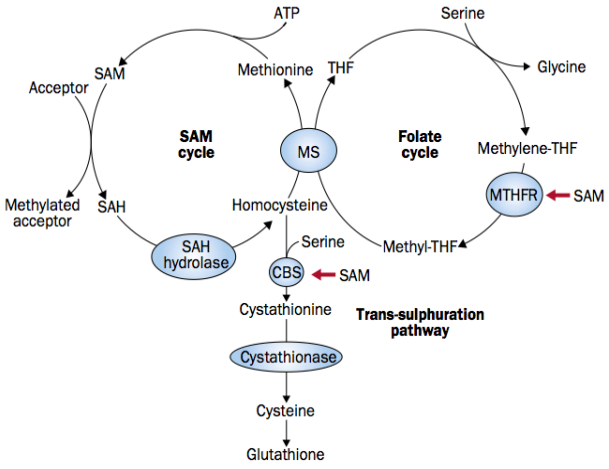

Figure 2. Homocysteine's role in one-carbon metabolism. *Adapted from Morris (2003).*

Studies have shown that increasing folate in the diet helps to reduce homocysteine levels in the plasma (Clarke, Frost, Leroy, & Collins, 1998). Folate is a substrate for the enzyme methionine synthase (MS), which is responsible for the

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conversion of homocysteine to methionine (Figure 2). Increased methionine production will subsequently result in increased S-adenosyl methionine (SAM) production. SAM excites the enzyme that synthesizes cystathionine from homocysteine, ultimately resulting in the lowering of homocysteine levels on two related fronts (Selhub & Miller, 1992).

The main strength of this study was the reliability and accuracy of the HPLC machine in measuring homocysteine and cysteine concentrations. HPLC has proven to be a rapid and reliable method to make these measurements. On the contrary, two weaknesses of this study were its relatively small sample size and its cross-section design, which does not permit detection of causative factors. Future research will increase sample size by completing evaluations of homocysteine and cysteine levels. Follow-ups of the MCI group will be conducted, as some patients in the group did have hyperhomocysteinemia (homocysteine > 10 μmol/L).

Conclusion

Alzheimer's disease is a major component of age-related dementia and requires much attention. Despite other studies finding an increased level of homocysteine in patients with AD, this study did not find significant differences in homocysteine and cysteine levels in patients with AD and MCI. A possible explanation for this disparity could be government-implemented folic acid fortification and the subsequent decrease in homocysteine concentrations due to increased dietary folate. Further research is needed to determine whether homocysteine is still a risk factor for AD in this era of folic acid fortification.

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