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DEMENTIAS - ORIGINAL ARTICLE

Elevated serum homocysteine level is not associated with serum C-reactive protein in patients with probable Alzheimer's disease

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Abstract Elevated plasma homocysteine (Hcy) levels have been associated with Alzheimer's disease (AD) and cognitive impairment. Studies have shown that Hcy may have direct and indirect neurotoxicity effects. The aim of the study was to investigate serum Hcy concentration in patients with probable AD with age-matched controls and to determine whether there was an association between serum Hcy and C-reactive protein concentration in patients with probable AD. We also aimed to determine whether there was an association between serum tHcy concentration and cognitive impairment in patients with probable AD. Serum concentration of total Hcy was determined by the fluorescence polarization immunoassay on the AxSYM system, and serum C-reactive protein (CRP) concentration was determined by means of particle-enhanced immunonephelometry with the use of BN II analyzer. Cognitive impairment was tested by the MMSE score. Body mass index (BMI) was calculated for each subject included in the study. Age, systolic and diastolic blood pressure and BMI did not differ significantly between the two groups. Mean serum tHcy concentration in the control group of subjects was 12.60 μmol/L, while in patients with probable AD the mean serum tHcy concentration was significantly higher than 16.15 μmol/L ($p < 0.01$). A significant negative association between serum tHcy concentration and cognitive impairment tested by the MMSE score in patients with probable AD was determined ($r = -0.61634$; $p < 0.001$). Positive, although not significant correlation between CRP and serum tHcy concentrations in patients with AD, was observed. Increased tHcy concentration in patients with probable AD, and the established negative correlation between serum tHcy concentration and cognitive damage tested by MMSE score in the same group of patients, suggests the possible independent role of Hcy in the pathogenesis of AD and cognitive impairment associated with this disease.

Keywords Alzheimer's disease · Homocysteine · C-reactive protein · Cognitive impairment

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Introduction

Alzheimer's disease (AD) is the most common cause of dementia, and it represents a clinical pathological state that literally means "loss of the ability to think". The profound biochemical and pathological alterations in the AD brain

result from cellular processes such as amyloid precursor protein (APP) and amyloid β-protein (Aβ) metabolism, tau-phosphorylation, oxidative stress, inflammation and lipid dysregulation (Gandy 2005; Zaciragic et al. 2007).

Homocysteine is a thiol-containing amino acid involved in the methionine cycle as the demethylation product of methionine and in the transulfuration pathway. Elevated Hcy is a risk factor for cardiovascular disease and seems to be an independent risk factor for AD. Hyperhomocysteinemia is associated with relative folate, vitamins B6 and B12 deficiencies, as well as with age, male sex, estrogen deficiency, renal insufficiency, and use of caffeine, dopamine agonist and anticonvulsant (Irizarry 2004). The pathophysiology of hyperhomocysteinemia and its possible role as AD risk factor is unknown (Irizarry 2004). Moreover, as shown in cell cultures, Hcy can directly cause brain damage through several mechanisms: increased glutamate excitotoxicity via activation of *N*-methyl-D-aspartate receptors, enhancement of β-amyloid peptide generation, impairment of DNA repair and sensitization of neurons to amyloid toxicity (Budge et al. 2000).

An association between AD and elevated tHcy concentrations has been reported in case–control (Clarke et al. 1998; McCaddon et al. 1998) and cross-sectional studies (Lehman et al. 1999; Quadri et al. 2004). Moreover, in the non-demented elderly population, plasma tHcy was inversely associated with poor performance of simultaneously performed tests of global cognitive function (Budge et al. 2000; Irizarry 2004; Ravaglia et al. 2003) and specific cognitive skills (Prins et al. 2002; Riggs et al. 1996).

C-reactive protein (CRP), a marker of low-grade chronic inflammation, is an acute phase reactant and is thought to be an important part of the innate immune system. CRP deposits have been immunohistochemically detected in the affected areas of the AD brain (Yaffe et al. 2003). Aβ peptide, amyloid P (AP) and CRP are powerful activators of complement cascade in senile plaques and neurofibrillary tangles of AD (McGeer and McGeer 2002). Through activation of the complement system, CRP may have an important role in an innate immune response of the brain. Complement cascade activation together with neuroinflammation may lead to neuronal loss, which provokes exacerbation of AD pathology. As an activator of the complement system, CRP may be a significant initiator of autodestructive inflammatory processes in brain and might represent an important target for pharmacological interventions in AD. It is still not fully elucidated whether accumulation of CRP and cytokines within the AD brain is followed with their increase in serum or plasma. Studies on serum CRP concentration in patients with AD have given discordant results (Gupta et al. 2005; Licastro et al. 2000).

Moreover, there has been very few studies, which aimed to assess possible association between Hcy and CRP in patients with Alzheimer's disease and/or cognitive impairment (Davis et al. 2007; Silbert et al. 2008). In vitro studies have shown that cytokines production increased in endothelial cells and macrophages treated with high homocysteine concentration. Based on these findings, it is possible to speculate that homocysteine might have an indirect effect on increased production of CRP (Poddar et al. 2001; Su et al. 2005).

Therefore, the aim of this study was to investigate serum Hcy concentration in patients with probable AD with age-matched controls and to determine whether there was an association between serum Hcy and CRP concentration in patients with probable AD.

Materials and methods

Patients and control group

Two groups of subjects were enrolled:

1. In this study, 30 patients (24 females and 6 males), aged 65 years and more, with clinically diagnosed probable AD by NINCDS-ADRDA criteria were included. All patients had a Mini Mental State Examination (MMSE) score of <23 (McKhann et al. 1984). The patients had Hachinski ischemic score of four or below (Loncarevic et al. 2005). We included all patients currently institutionalized at specialized units for patients with dementia, within the health-care hospice for persons with disabilities and other persons in Sarajevo, Bosnia and Herzegovina.
2. Furthermore, in this study, 30 community-dwelling, age-matched apparently healthy (22 females and 8 males), asymptomatic, controls without dementia were included. All subjects in this group had an MMSE score of >26.

For both groups of subjects, exclusion criteria were a positive history of cardiovascular or thyroid disease, chronic inflammatory disease (asthma and rheumatoid arthritis), hepatic or renal insufficiency, cancer and vascular dementia.

Approval for the study was obtained by the local ethics committee. All procedures on human subjects were performed according to the Declaration of Helsinki, 1975. Informed consent was obtained from subjects and caregivers. Subjects underwent a history, a clinical examination and a Mini Mental State Examination.

Blood analysis

Non-fasting blood samples were drawn from the antecubital vein into siliconized tubes. After venipuncture,

Table 1 Baseline characteristics of control subjects (C) and patients with probable AD

	AD group MMSE	Control group MMSE	*p* Value
Age (years) (mean ± SEM)	79.96 ± 0.95	77.53 ± 0.96	NS
Sex (% female)	80	73.3	NS
SBP (mmHg) (mean ± SEM)	133.23 ± 3.49	130.13 ± 3.12	NS
DBP (mmHg) (mean ± SEM)	82.66 ± 1.58	82.30 ± 1.46	NS
BMI (kg/m^2) (mean ± SEM)	23.71 ± 1.03	25.31 ± 0.54	NS
MMSE score (mean ± SEM)	4.53 ± 0.62	28.33 ± 0.25	0.0001

SBP systolic blood pressure, *DBP* diastolic blood pressure, *BMI* body mass index, *MMSE* Mini Mental State Examination

blood samples were put on ice. The median time between venipuncture and centrifugation in our laboratory was 50 min (interquartile range: 30–70 min). Serum samples were stored at or below −20°C. Serum tHcy concentration was determined by using a fluorescence polarization immunoassay on the AxSYM system (Pernet et al. 2000). The reference interval for tHcy concentration with the use of this method is from 3.36 to 20.44 μmol/L.

Serum CRP concentration was determined by means of particle-enhanced immunonephelometry with the use of BN II analyzer at the Institute for Chemistry and Biochemistry, Clinical Centre of the University of Sarajevo. CardioPhase high-sensitivity CRP (DADE BEHRING) was used as a diagnostic reagent. It consists of a suspension of polystyrene particles coated with mouse monoclonal antibodies to CRP. Reference interval for CRP with the use of this method is from 0 to 5 mg/L.

Statistical analyses

Statistical analyses were performed with Microsoft Office Excel 2003 and SPSS, version 12.0. Data are presented as mean ± SEM. Data distribution was determined using the Kolmogorov–Smirnov test. Difference for normally distributed variables was tested with Student *t* tests or Mann–Whitney test where appropriate. Additionally, Pearson correlations were used as measures of association for the continuous variables. Statistical significance was set at $p < 0.05$.

Results

The baseline characteristics of two groups enrolled in the study are reported in Table 1. No differences emerged in age, and systolic and diastolic blood pressure between the groups. No difference in BMI was found between the two groups. Subjects with probable AD had statistically significantly lower MMSE scores compared with the C ($p < 0.0001$).

As shown in Fig. 1 and Table 2, patients with probable AD had significantly higher serum tHcy concentration compared to the C ($p < 0.01$).

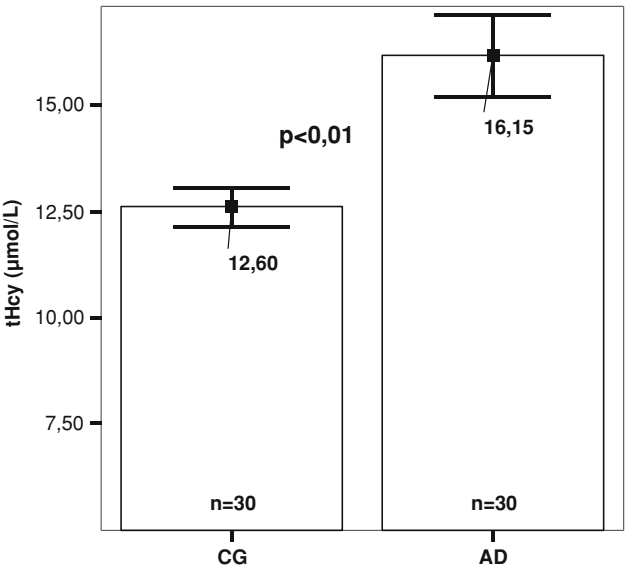

Fig. 1 Mean serum total homocysteine concentration in control and AD group. *Bars* show means and *error bars* show mean ± SEM; *tHcy* total homocysteine, *CG* control group, *AD* Alzheimer's disease group

Table 2 Total serum homocysteine in C and and patients with probable AD

Total Hcy C ($n = 30$) (μmol/L)	Total Hcy AD ($n = 30$) (μmol/L)
12.60 ± 0.44	16.15 ± 0.96

Data are presented as mean ± SEM

Figure 2 shows significantly negative correlation between serum tHcy concentrations and cognitive impairment, determined by MMSE in patients with probable AD ($r = -0.616$; $p < 0.001$).

As shown in Table 3, female patients with probable AD had significantly higher serum tHcy concentrations as compared to the C group ($p < 0.01$).

Mean serum CRP concentration was not significantlly higher in patients with probable AD compared to control subjects (3.36 ± 0.61 vs. 2.31 ± 0.29 mg/L). Female and male patients with probable AD did not have significantlly higher serum CRP concentrations compared to the C group

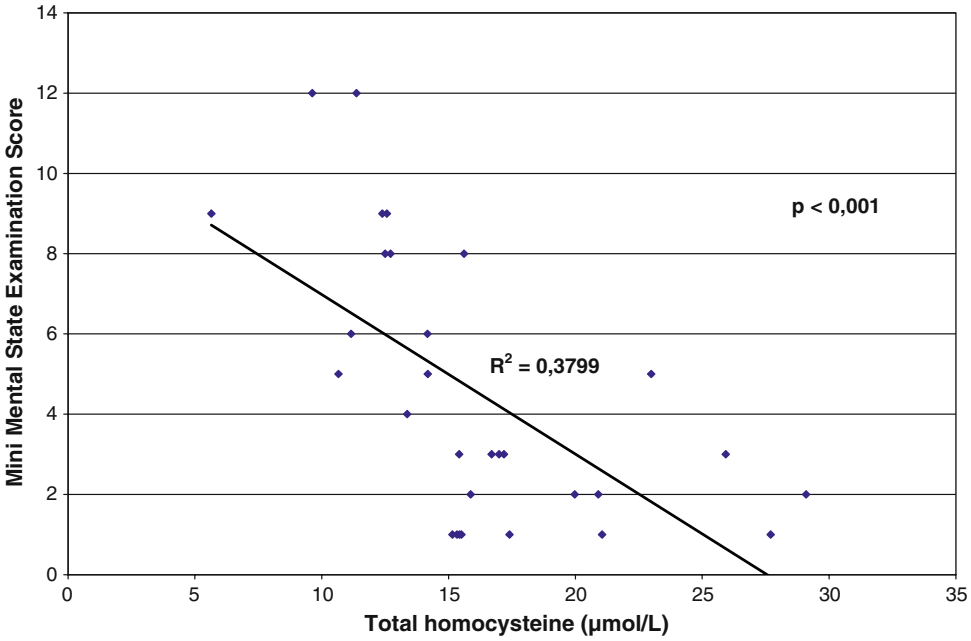

Fig. 2 Correlation between serum total homocysteine concentrations and cognitive impairment tested by MMSE score in the AD group

(3.9 ± 0.72 vs. 3.23 ± 0.6 mg/L and 1.2 ± 0.43 vs. 1.05 ± 0.14 mg/L, respectively).

As presented in Table 4, positive, although not significant, correlation between CRP and serum tHcy concentrations in patients with AD was observed.

Discussion

Previous cross-sectional studies have found elevated Hcy levels in patients with AD (Clarke et al. 1998; McCaddon et al. 1998). The relationship between increased Hcy level and AD may be explained via several mechanisms. Homocysteine has a direct neurotoxic effect and is associated with cerebrovascular disease (CVD), which is currently believed to play a significant role in AD etiology. Moreover, elevated concentrations of plasma tHcy are an indicator of inadequate folate and vitamin B12 status and can directly affect brain function via altered methylation reactions (Ravaglia et al. 2005).

Seshadri et al. (2002) found that elevated plasma Hcy levels are an independent risk factor for AD. These authors suggest that a plasma Hcy level of 5 μmol/L increases the risk of AD by 40%. Their results showed that the observed association between increased plasma Hcy level and AD was independent of age, sex, apolipoprotein E genotype, plasma vitamin levels and other putative risk factors for dementia and AD.

The main finding of this study is a significantly higher serum tHcy concentration in patients with probable AD compared with the control group. Our observations are consistent with those of McCaddon et al. (1998) who also found elevated serum concentration levels of Hcy in patients with AD compared with the control group of subjects. Another British case–control trial within the OPTIMA project involved 164 patients with dementia. The results of this trial showed that tHcy was higher, and both serum folate and vitamin B12 lower, in patients with dementia of Alzheimer's type compared to the control group of subjects. The authors suggest that the low vitamin levels and high tHcy levels either existed before the start of AD or developed early in the disease phase (Clarke et al. 1998). Either way, the abnormality in these biochemical markers may be relevant to the clinical course of AD and

Table 3 Total serum homocysteine concentration in males and females in AD and C group

Sex	AD group	Control group	*p* Value
Female			
Total Hcy (μmol/L)	15.99 ± 1.05	12.54 ± 0.54	0.008
Male			
Total Hcy (μmol/L)	16.775 ± 2.55	12.75 ± 0.77	0.053

Data are presented as mean ± SEM

Table 4 Correlation analysis between serum C-reactive protein (*CRP*), body mass index (*BMI*) and serum total homocysteine concentration in control subjects (C) and patients with probable AD

	AD group total Hcy	Control group total Hcy
BMI (kg/m^2)	$r = -0.14$	$r = 0.02$
CRP (mg/L)	$r = 0.19$	$r = 0.17$

CRP C-reactive protein, *BMI* body mass index, r-correlation coefficient

should be considered in clinical trials as possible targets for therapeutic intervention.

A recent Swedish study involving patients with early (EOAD) and late (LOAD) onset of AD showed that there was no difference in tHcy or its determinants between the EOAD group and age- and sex-matched controls. In contrast, patients with vascular dementia (VAD) or mixed AD/VAD showed increased tHcy. Total Hcy was also elevated in patients with LOAD and a history of CVD compared with both AD patients without a history of CVD and with controls. These findings suggest that elevated tHcy contributes to dementia mainly through vascular mechanisms (Nilsson et al. 2002).

Zhang et al. found that hyperhomocysteinemia could increase Aβ production through the enhanced expression of secretase and APP phosphorylation, causing memory deficits that could be prevented by folate and vitamin B12 treatment in rats. The authors suggest that hyperhomocysteinemia may serve as an upstream factor for increased Aβ production as seen in patients with Alzheimer's disease (Zhang et al. 2009).

We found a significant negative association between serum tHcy concentration and cognitive impairment tested by MMSE scores in patients with probable AD. Our observations are consistent with those of Leblhuber et al. (2000) who also found significant inverse correlation between the degree of cognitive impairment and tHcy in a study of 19 patients with AD and 12 with vascular dementia. In view of the latter finding, it is of interest that elevated Hcy concentrations are associated with a more rapid rate of atrophy of the medial temporal lobe in patients with Alzheimer's disease. Moreover, in a healthy elderly cohort, the thickness of the medial temporal lobe is inversely related to Hcy concentrations (Smith 2002). Conversely, Kalmijn et al. (1999) found no significant association between tHcy and cognitive impairment in a sample of 630 subjects, 55 years and older, who were participants in a cross-sectional, population-based Rotterdam Study.

Our results did not show any association between serum total homocysteine concentrations and CRP in patients with probable AD. These data suggest that Hcy may be an independent risk factor in the pathogenesis of AD.

Homocysteine may simply be a marker of low concentrations of vitamins that are its main biological determinants (folate and vitamin B12). Indeed, low concentration of folate is associated with brain atrophy at autopsy in subjects with AD. Another possibility is that Hcy itself is the damaging agent; Hcy has been shown to be neurotoxic, leading to DNA damage and apoptosis. Furthermore, hyperhomocysteinemia in patients with Alzheimer's disease is associated with activation of the cell cycle in hippocampal neurons, a plausible precursor of apoptosis and of AD type of pathology (Smith 2002). The finding that vascular dementia, as well as AD, is associated with hyperhomocysteinemia raises the possibility that the brain suffers a double setback from elevated concentrations of Hcy: cerebrovascular damage that triggers or potentiates the effect of AD-type pathology together with a direct neurotoxic effect from Hcy. Zhang et al. (2008) showed that homocysteine may be an upstream effector to induce AD-like tau hyperphosphorylation through inactivating PP2A.

Increased Hcy concentration in patients with probable AD and established negative correlation between serum Hcy concentrations and cognitive damage tested by MMSE scores, observed in our study, suggest a possible role for Hcy in the pathogenesis of AD and cognitive impairment. These findings imply that the serum Hcy level may be a potential biomarker for AD, as well as a marker of cognitive damage associated with this disease.

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