Neuroendocrinology Letters Volume 35 No. 1 2014
ISSN: 0172-780X; ISSN-L: 0172-780X; online ISSN: 2354-4716

ORIGINAL ARTICLE

Plasma homocysteine in Alzheimer's disease with or without co-morbid depressive symptoms

Eva Kitzlerová [1], Zdeněk Fišar [1], Roman Jirák [1], Martina Zvěřová [1], Jana Hroudová [1], Hana Benáková [2], Jiří Raboch [1]

1 Department of Psychiatry, Charles University in Prague and General University Hospital in Prague, Czech Republic
2 Institute of Medical Biochemistry and Laboratory Diagnostics, Charles University in Prague and General University Hospital in Prague, Czech Republic

Correspondence to: Prof. Zdeněk Fišar, PhD.
Department of Psychiatry, First Faculty of Medicine,
Charles University in Prague and General University Hospital in Prague
Ke Karlovu 11, 120 00 Prague 2, Czech Republic.
tel: +420-224965313; e-mail: zfisar@lf1.cuni.cz

Submitted: 2014-01-03 Accepted: 2014-01-22 Published online: 2014-02-27

Key words: **Alzheimer's disease; depressive disorder; homocysteine; cognitive impairment**

Neuroendocrinol Lett 2014; **35**(1):42–49 **PMID:** 24625917 NEL350114A05

Abstract

OBJECTIVE: Elevated homocysteine is associated with a variety of diseases, including Alzheimer's disease (AD) and depressive disorder. This study was designed to detect an association between plasma homocysteine and AD with or without co-morbid depressive symptoms.
METHODS: Plasma homocysteine concentrations were measured in 85 AD patients (36 of them with depressive symptoms), 33 non-AD patients with a depression diagnosis and 44 healthy controls, all aged above 50 years.
RESULTS: Positive correlation between age and homocysteine was confirmed. Significantly higher mean plasma homocysteine was found in AD patients, but not in depressive patients, when compared with controls. We confirmed significant correlation between homocysteine concentration and the degree of cognitive impairment in AD patients. There was no incremental effect of concurrent depressive symptoms on homocysteine concentration in AD patients.
CONCLUSION: The association of high homocysteine with degree of cognitive impairment or stage of dementia in AD indicate potential role of high plasma homocysteine as a biomarker of the disease and/or indicator of brain damage during the progression of AD dementia.

Abbreviations:

AD	- Alzheimer's disease
CGI-S	- Clinical Global Impression - Severity scale
GDS	- 15-item Geriatric Depression Scale
HRSD-21	- 21-item Hamilton Rating Scale for Depression
MMSE	- Mini-Mental State Examination
OR	- odds ratio
RR	- relative risk

INTRODUCTION

Elevated total homocysteine concentrations are associated with a variety of diseases (Refsum *et al.* 2006), including neurodegenerative and psychiatric disorders, such as Alzheimer's disease (AD) (Seshadri *et al.* 2002; Van Dam & Van Gool 2009; Cankurtaran *et al.* 2013), depressive disorder

(Tolmunen *et al.* 2004; Almeida *et al.* 2008; Nabi *et al.* 2013) and schizophrenia (Kale *et al.* 2010; Bicikova *et al.* 2011). However, previous studies have shown conflicting results. Homocysteine is both a marker of folate (vitamin B_9) or vitamin B_{12} deficiency (Bottiglieri *et al.* 2000) and a cause of many adverse effects on neurons resulting in disturbed biosynthesis of neurotransmitters and neurodegenerative damage (Mattson & Shea 2003). Changes in homocysteine metabolism, inadequate intake of vitamin B complex, impaired renal function, increasing age, male sex, smoking, excessive alcohol intake, lack of physical activity and high coffee consumption lead to increased total homocysteine blood levels (Bottiglieri 2005; Refsum *et al.* 2006).

Cellular mechanisms involved in neurodegenerative diseases include oxidative stress, over-activation of glutamate receptors, mitochondrial dysfunctions, metabolites of polyunsaturated fatty acids, activation of apoptosis and neuronal death (Obeid & Herrmann 2006; Hroudová & Fišar 2011; Zeman *et al.* 2012). A stressful event in the elderly could potentially trigger a cognitive decline (Tsolaki *et al.* 2010). Homocysteine can promote glutamate excitotoxicity through overstimulation of *N*-methyl-D-aspartate receptors (Lipton *et al.* 1997), DNA damage and activation of apoptosis (Mattson & Shea 2003). Thus, homocysteine can contribute to neuronal degeneration in age-related or stress-related neuropsychiatric disorders. Diseases of the central nervous system, such as AD, vascular dementia, cognitive impairment or stroke, were found in patients with severe hyperhomocysteinemia (Hermann & Obeid 2011).

Depressive disorder

It was shown in several large studies that elevated plasma or serum homocysteine concentrations and folate and vitamin B_{12} deficiency are all significantly associated with depressive disorders (Tiemeier *et al.* 2002; Gu *et al.* 2012) or with increased risk of depression (Tolmunen *et al.* 2004; Refsum *et al.* 2006; Gilbody *et al.* 2007; Almeida *et al.* 2008). Approximately one third of patients with a depression diagnosis showed low concentrations of folate and elevated concentrations of homocysteine in serum or erythrocytes (Carney *et al.* 1990; Bottiglieri *et al.* 2000). Moreover, several studies have supported the use of folate and vitamin B_{12} supplementation in the treatment of depression (Bottiglieri 2005; Fava & Mischoulon 2009; Lazarou & Kapsou 2010).

The vascular depression hypothesis proposes that cerebrovascular disease can predispose, precipitate or perpetuate some geriatric depressive syndromes (Alexopoulos *et al.* 1997). The homocysteine hypothesis of depression presumes that genetic and environmental factors elevate homocysteine levels, which cause vascular disease of the brain and/or neurotransmitter alterations, which in turn cause depression (Folstein *et al.* 2007). However, another important pathophysiological mechanism should be taken into account when discussing the homocysteine hypothesis of depression, namely the role of homocysteine and its metabolites in DNA methylation (Miller 2008) or in gene-environment interactions. These processes play a role in the pathogenesis of different psychiatric disorders, not only depression but also dementia, schizophrenia, eating disorders and addiction.

Alzheimer's disease

The current data suggests that there is a positive association between plasma concentrations of homocysteine and amyloid beta deposition in the brain in neurodegenerative disease (Obeid & Herrmann 2006). Prospective studies have shown that elevations of plasma homocysteine precedes the development of dementia and that there is an inverse linear relationship between plasma homocysteine concentrations and cognitive performance in the elderly, i.e. increased plasma homocysteine concentration is associated with increased risk for the development of dementia and AD. However, it is not clear whether homocysteine concentration is a risk factor or risk marker (Seshadri *et al.* 2002; Ravaglia *et al.* 2005; Seshadri 2006; Zhuo *et al.* 2011). Increased total homocysteine or progressive increase of its concentration is supposed to be one of the risk factors for cognitive impairment in the elderly and/or AD (Hooshmand *et al.* 2010, 2012), and homocysteine-lowering therapy may slow AD progression (Connelly *et al.* 2008; Smith *et al.* 2010; deJager *et al.* 2012).

Depression belongs to co-morbid diseases without significant differences in prevalence between AD and control subjects (Heun *et al.* 2013). Depressive symptoms in the elderly are mostly symptoms of genuine depression instead of prodromes of AD (Mossaheb *et al.* 2012). Nevertheless, depressive symptoms exacerbate pre-existing patient morbidity in up to 50% of AD patients (Heun *et al.* 2002; Lyketsos & Olin 2002; Starkstein *et al.* 2005; Lyketsos *et al.* 2011; Spalletta *et al.* 2012).

Aims of study

We aimed to examine the association of plasma homocysteine with the disease in AD patients with or without current depressive symptoms, and in elderly depressive patients without AD. The study attempts to analyse association between plasma homocysteine and scores of diagnostic questionnaires characterizing cognitive impairment and severity of depression. The results are assessed also in terms of applicability of high plasma homocysteine as a marker of AD.

MATERIAL & METHODS

Subjects

Patients with the ICD-10 diagnosis of depressive disorder and patients with the diagnosis of AD were recruited from the Department of Psychiatry of the First Faculty of Medicine and General University Hospital in Prague.

The patients were asked to complete a data set relating to medical history, personal habits and use of medication. Folate and vitamin B_{12} supplementation was not used in their treatment. Global cognitive impairment in AD patients was screened by Mini-Mental State Examination (MMSE) and depressive symptoms, which are included in behavioural and psychological symptoms of AD dementia, were assessed by 15-item Geriatric Depression Scale (GDS) (Yesavage *et al.* 1982–83; Mitchell *et al.* 2010).

The inclusion criteria for AD patients were as follows: age >50 years; diagnosis of probable AD according to the Alzheimer's Criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA; McKhann *et al.* 1984); structural brain imaging (magnetic resonance imaging or computed tomography) showing corticosubcortical atrophy (atrophy in the hippocampus and temporal lobe); MMSE score <26 (score below 26 in context with clinical picture and brain atrophy indicates possible dementia); no serious unstable somatic disease; and brain imaging excluding vascular changes, intracranial haemorrhage and other cause of dementia. Other causes of dementia were excluded, including pseudo-dementia (behavioural changes that resembles those of the progressive degenerative dementias, but without *de facto* impairment of cognitive functions). AD patients with GDS ≥7 were sub-grouped as those with clinically relevant depressive symptoms.

Severity of depression was assessed using the 21-item Hamilton Rating Scale for Depression (HRSD-21) and Clinical Global Impression – Severity scale (CGI-S) in patients with depressive disorder without AD. Inclusion criteria for participants with depressive disorder included age above 50 years and current depressive episode without psychotic symptoms, with single or recurrent depressive episode. Diagnoses of depressive episode (F32) or recurrent depressive episode (F33) were confirmed by structured clinical interview for ICD-10. Serious somatic disease or chronic somatic pharmacotherapy was not present and patients were without organic brain disease, without cognitive impairment and without abuse of psychoactive substances. Depressed patients had to have the HRSD score greater than 10 and the CGI-S score equal to 2 or more. A negative screen for bipolar disorder was found for all tested subjects using the Mood Disorder Questionnaire.

The control group consisted of normal healthy volunteers above 50 years of age. These volunteers underwent a psychiatric examination in the same way as AD patients and qualified as non-demented, non-depressed and without any organic brain disorder.

The study was carried out according to the principles expressed in the Declaration of Helsinki and the study protocol was approved by the Ethical Review Board of the First Faculty of Medicine and General University Hospital in Prague. Written informed consent was obtained from all the subjects.

Plasma homocysteine measurement

Fasting blood samples were taken between 7:00 and 8:00 am for the determination of total plasma homocysteine. Vacutainer® blood collection tubes were used with EDTA as anticoagulant. Plasma was separated immediately after blood sampling and samples were stored at –70 °C until the time of analysis. Total plasma homocysteine was assayed spectrophotometrically using the liquid stable 2-part homocysteine reagent kit (Axis-Shield Diagnostics Ltd, Dundee, UK) and analyser Modular Analytics EVO (Hitachi, Japan).

Statistical analysis

Statistical analyses were performed using the STATISTICA data analysis software system (StatSoft, Inc., Tulsa, OK, USA). All data are presented as the mean ± standard deviation except for plasma homocysteine. The normality of the data was tested by Shapiro-Wilk's W test. Both plasma homocysteine and logarithm of plasma homocysteine did not fulfil criteria of normal distribution. Hypothesis that distribution of logarithm of logarithm of plasma homocysteine concentrations is normal distribution was not rejected for all tested groups of participants; thus, parametric statistics was used for analysis of log(log)-transformed data. Because of the skewed distribution of homocysteine concentrations, mean values and SD-derived error intervals were determined by inverse transformation of log(log(mean ± SD)).

General linear models were used for investigation whether there are significant interaction effects for the plasma homocysteine and both categorical and continuous predictor variables; i.e. analysis of covariance (ANCOVA) was used to evaluate whether population means of plasma homocysteine are equal across levels of a categorical independent variable (gender), while statistically controlling for the effects of other continuous variables that are not of primary interest. Partial correlation, a correlation between two variables that remains after controlling for one or more other variables, was calculated to measure the degree of association between plasma homocysteine and clinical variables.

Quartiles, values that divide the data set into four equal groups, each representing a fourth of the population being sampled, were calculated from the data of the control group. Relative number of AD or depressive patients in each quartile was determined and used to clarify the association between the disease and plasma homocysteine. The "high homocysteine group" was selected for homocysteine concentrations above 15 μmol/L (Refsum *et al.* 2006), and odds ratio (OR), relative risk (RR), sensitivity and specificity, all with 95% confidence interval (95% CI), were calculated to estimate the association of homocysteine status with AD and/or depression. All of these analyses were adjusted for age.

RESULTS

Plasma homocysteine was measured in blood samples of patients with AD (n=85), elderly non-AD patients with depressive disorder (n=33), and elderly controls (n=44). AD patients were sub-grouped as follows: (1) AD patients with clinically relevant depressive symptoms (GDS ≥7; n=36) and (2) AD patients without depressive symptoms (GDS <7; n=49). Among the AD patients, 40 of them suffered by mild dementia and MMSE >20, 33 of them by moderate dementia and 21> MMSE >9, and 12 of them by severe dementia and MMSE<10. Demographic data of participants, clinical assessment and plasma homocysteine concentrations are summarized in Table 1.

While slightly higher mean plasma homocysteine concentrations were found in men compared with women in all tested groups, statistical analysis (ANCOVA and post-hoc Scheffé test; controlled for the age) did not discover any significant difference for all AD (p=0.070), AD with depressive symptoms (p=0.15), AD without depressive symptoms (p=0.30), depressive disorder without AD (p=0.32), and controls (p=0.99), respectively. Thus, the data from women and men were analyzed in common in our study.

In the control group we confirmed association between plasma homocysteine and the age; significant partial correlation controlled for the gender was found (ρ=0.370, n=44, p=0.014). Therefore, adjustment of plasma homocysteine for age was performed in all analyses. Figure 1 displays the data for relationship between the age and plasma homocysteine concentration in controls.

Significantly higher mean plasma homocysteine concentration (adjusted for age) was found in the group of all AD patients (p=0.0030) and in subgroup of AD patients without depressive symptoms (p=0.00075) when compared with controls (Table 1). Significantly increased plasma homocysteine concentration (adjusted for age) was found also in AD patients with moderate to severe stage of dementia and MMSE ≤20 compared with both controls (p=0.000092) and AD patients with mild stage of dementia and MMSE >20 (p=0.025).

Association was tested of plasma homocysteine (1) with MMSE and GDS in AD patients, and (2) with

Tab. 1. Demographic and clinical data, and plasma homocysteine concentrations.

Characteristic	all	Alzheimer's disease				Depressive disorder	Control
		with mild dementia	with moderate to severe dementia	with depression	without depression		
Age (years)	***75.6±7.7	***73.7±7.8	***77.3±7.2	***76.1±6.7	***75.2±8.4	59.2±5.8	63.0±7.7
	(56-91)	(56-88)	(61-91)	(62-87)	(56-91)	(51-74)	(51-77)
Education (years)	13.9±2.8	14.4±2.7	13.4±2.8	14.0±2.9	13.8±2.7	13.4±2.1	13.9±1.9
	(8-18)	(9-18)	(8-18)	(8-18)	(9-18)	(9-18)	(12-18)
BMI (kg/m^2)	**24.1±3.4	**23.8±3.2	**24.2±3.6	25.6±4.0	***22.9±2.4	26.3±5.3	27.8±3.4
	(18.7-36.3)	(20.4-36.3)	(18.7-36.3)	(18.7-36.3)	(19.6-30.2)	(18.0-33.9)	(22.4-34.0)
GDS	***6.0±3.6	***6.0±3.7	***5.9±3.5	***9.7±2.1	***3.2±1.4	—	0.3±0.8
	(1-13)	(1-13)	(1-12)	(7-13)	(1-6)		(0-4)
MMSE	***18.8±6.9	***24.1±2.3	***14.1±6.2	***19.7±6.5	***18.2±7.2	29.4±1.1	29.5±0.9
	(1-25)	(21-25)	(1-20)	(1-25)	(2-25)	(26-30)	(26-30)
HRSD	—	—	—	—	—	24.4±7.9	—
						(10-41)	
CGI-S	—	—	—	—	—	4.3±1.2	—
						(1-6)	
Homocysteine (μmol/L)	**15.9 [11.0-24.3]	14.5 [10.5-20.9]	***17.4 [11.7-27.5]	15.1 [9.9-24.7]	***16.6 [12.1-23.8]	13.1 [9.3-19.5]	13.1 [10.6-16.4]
	(6.0-41.2)	(8.0-29.4)	(6.0-41.2)	(6.0-41.2)	(8.4-37.1)	(7.3-59.4)	(8.4-27.9)
n (women/men)	85 (51/34)	40 (24/16)	45 (27/18)	36 (22/14)	49 (29/20)	33 (25/8)	44 (31/13)

Data is displayed as mean ± SD (range), except for homocysteine. Mean value and SD was calculated for transformed log(log(homocysteine)) and displayed values (with SD-derived error interval in square brackets) were determined by inverse transformation. ANOVA and post-hoc Dunnett test were used to determine indicated p-level when compared to controls, whereas homocysteine data was adjusted for age; * p<0.05, ** p<0.01, *** p<0.001.
BMI – body mass index; GDS – 15-item Geriatric Depression Scale; MMSE – Mini-Mental State Examination; HRSD – 21-item Hamilton Rating Scale for Depression; CGI-S – Clinical Global Impression – Severity scale.

HRSD score and CGI-S score in non-AD patients with depressive disorder. Control was performed for demographic variables, age, gender, years of education and body mass index (BMI). Analysis exploiting the general linear models did not discover any significant regression coefficients in the group of depressive patients; i.e. none of the independent variables contributed significantly to the prediction of plasma homocysteine in this group. However, we found significant association of plasma homocysteine with the MMSE score in AD patients (Table 2). The relationship between plasma homocysteine concentration in AD patients and the MMSE or GDS score was documented by partial correlations and *p* values for model unadjusted, adjusted for age and gender, and adjusted for age, gender, BMI, and years of education. The MMSE was significantly associated with plasma homocysteine in the whole group of AD patients both with and without adjustment for demographic variables. Note, that *p* values were slightly increased after adjustment for confounding factors. The correlation between plasma homocysteine and MMSE score in AD patients is shown on the Figure 2.

There was lesser range of plasma homocysteine concentrations in controls compared with other groups (Table 1). To determine if both low and high plasma homocysteine concentrations are associated with AD or depressive disorder, we calculated relative number of patients in quartiles derived from controls. Relative number of patients in each quartile is displayed on Figure 3, which shows that the association between plasma homocysteine and both AD and depression is U-shaped, with increased relative number of patients with high homocysteine. Note that both quartiles and relative number of patients in several quartiles were calculated with plasma homocystein concentrations adjusted for age.

The previous analysis was extended for subgroups of AD patients and quantified using the odds ratio, relative risk, sensitivity, and specificity in the subjects with age-adjusted high plasma homocysteine concentrations (>15 μmol/L at age 50 years). High homocysteine concentrations (adjusted for age) were found in 34.1% (*n*=29) of all AD patients, 32.7% (*n*=16) of AD without depressive symptoms, 36.1% (*n*=13) of AD with depressive symptoms, 22.5% (*n*=9) of AD with MMSE >20 and mild dementia, 44.4% (*n*=20) of AD with MMSE ≤20 and moderate to severe dementia, 19.2% (*n*=6) of depressive patients, and 9.1% (*n*=4) of controls.

The following relationships were analysed: (1) AD among subjects with AD and control subjects; (2) AD without depressive symptoms (GDS <7) among these and control subjects; (3) AD with depressive symptoms (GDS ≥7) among these and control subjects; (4) AD with MMSE >20 among these and controls; (5) AD with MMSE ≤20 among these and controls; (6) Depressive patients among subjects with depressive disorder and control subjects (Figure 4).

Test sensitivity was low both for AD patients (0.34) and for depressive patients (0.25) when specificity 0.91 was determined from values of the control group for

Tab. 2. Relationships between plasma homocysteine concentration, Mini-Mental State Examination (MMSE) score and Geriatric Depression Scale (GDS) in patients with Alzheimer's disease.

Model		MMSE	GDS
A	Partial correlation	*-0.275	-0.041
	p-value	0.011	0.709
B	Partial correlation	*-0.237	-0.070
	p-value	0.032	0.533
C	Partial correlation	*-0.233	-0.161
	p-value	0.038	0.156

Model A: Unadjusted.
Model B: Adjusted for age and gender.
Model C: Adjusted for age, gender, education, and body mass index.
Significance level: * $p<0.05$.

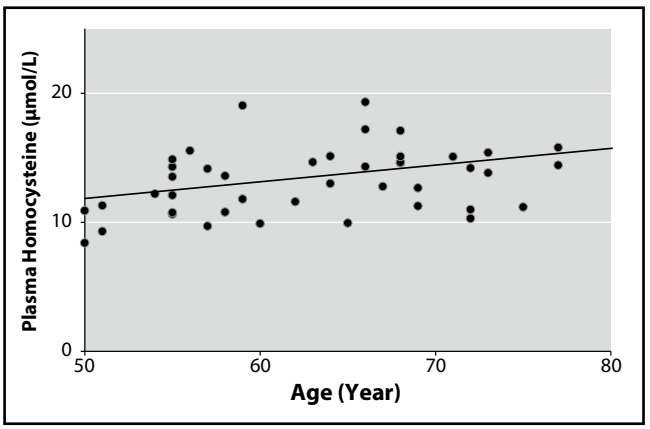

Fig. 1. The plot of plasma homocysteine concentrations in controls against age. Significant partial correlation controlled for the gender was found equal to 0.370 (p=0.014) for controls aged above 50 years.

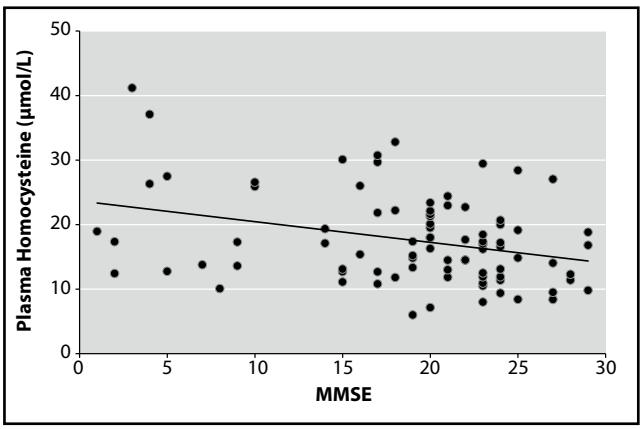

Fig. 2. The linear regression plot of a plasma homocysteine in patients with Alzheimer's disease (AD) against Mini-Mental State Examination (MMSE) score.

lower limit of high homocysteine equal to 15 μmol/L. Significantly increased odds ratio (Figure 4) and relative risk of disease in persons with high plasma homocysteine was found for AD patients (RR=1.51, 95% CI 1.15–1.73), the subgroup of AD patients without depressive symptoms (RR=1.77, 95% CI 1.14–2.24), the subgroup of AD patients with depressive symptoms (RR=2.10, 95% CI 1.22–2.84) and AD patients with moderate to severe dementia and MMSE ≤20 (RR=2.17, 95% CI 1.43–2.74).

DISCUSSION

This study was designed to analyse an association of AD and/or depression with plasma homocysteine concentration. We aimed to determine applicability of high plasma homocysteine as a marker of cognitive impairment or stage of dementia in AD. Obtained data supported the role of high homocysteine as member of a panel of biomarkers of AD.

Our data confirmed previous reports that there is an increase in homocysteine concentration in elderly persons as well as in elderly patients with mental disorders (Refsum *et al.* 2006). We found significant positive correlation between the age and plasma homocysteine levels in healthy controls (Figure 1). Significantly increased mean plasma homocysteine concentration was found in the AD patients but not in depressed patients (Table 1). Our data confirmed that both AD and depressive disorder is associated with increased plasma homocysteine (Figure 3).

The finding that high plasma homocysteine is associated with AD, was confirmed by our data. The analysis showed association of plasma homocysteine with MMSE score in AD patients (Table 2), but no association was observed of plasma homocysteine with clinical parameters in patients with depressive disorder without AD. Significant association was found between plasma homocysteine and MMSE in whole group of AD patients both using unadjusted model and after adjustment for age, gender, education, and BMI. Because GSD score was not significantly associated with plasma homocysteine, we suppose that plasma homocysteine reflect MMSE-assessed degree of cognitive impairment in AD rather than severity of co-morbid depression.

To express the utility of increased plasma homocysteine concentration in a panel of biological markers of the disease, we dichotomized the data to those with "normal" and "high" plasma homocysteine. Association between high plasma homocysteine and AD was quantified by calculation of odds ratio or relative risk and 95% confidence interval. Significantly increased odds ratio or relative risk of disease in subjects with high plasma homocysteine was found in all AD, in AD with depressive symptoms, in AD without depressive symptoms and in AD with moderate to severe stage of dementia especially, but not in AD with mild stage of dementia or in non-AD patients with depressive disorder (Figure 4). The results indicate that there is no simple association between plasma homocysteine levels and clinical and cognitive assessments when depressive symptoms are present.

Despite the small sample size in this study, our preliminary results indicate that higher homocysteine levels are associated with cognitive impairment quantified by the MMSE score in AD patients. The data are

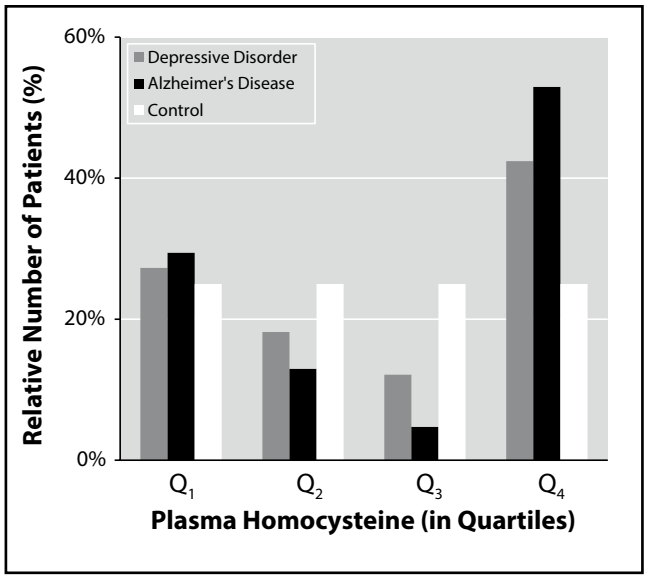

Fig. 3. Distribution of relative number of patients with depressive disorder or Alzheimer's disease according to plasma homocysteine concentration. Following quartiles determined for the control group after adjustment for age were used: Q_1=homocysteine<11.70, Q_2=11.70 ≤ homocysteine <13.09, Q_3=13.09≤ homocysteine <14.61, Q_4=homocysteine ≥14.61 μmol/L

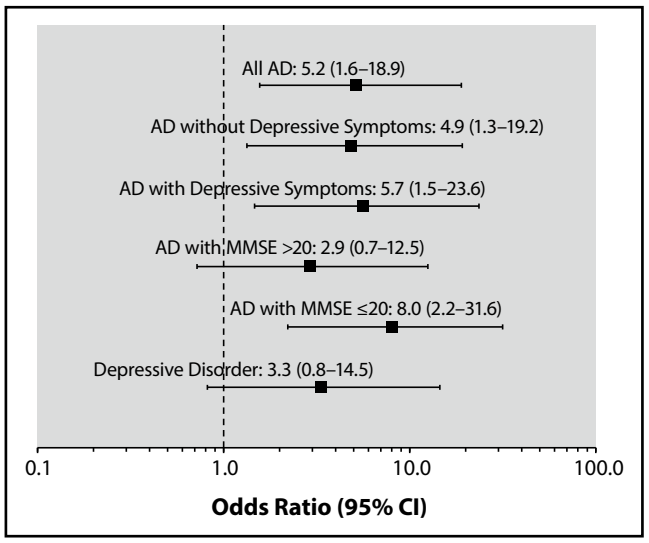

Fig. 4. Odds ratios (95% Confidence Intervals, CI) for association between high plasma homocysteine (>15 μmol/L at age 50 years) and Alzheimer's disease (AD), AD without depressive symptoms, AD with depressive symptoms, AD with mild dementia and Mini-Mental State Examination (MMSE) score >20, AD with moderate to severe dementia and MMSE ≤20, and depressive disorder.

consistent with earlier reports that elevated homocysteine levels correlate with cognitive impairment in AD (Hooshmand *et al.* 2010, 2012) and that plasma homocysteine could reflect progression of the AD.

We observed that co-morbid depressive symptoms in AD patients are not significantly associated with plasma homocysteine concentration, notwithstanding severity of dementia, i.e. there was no incremental effect of co-morbid depression in AD on plasma homocysteine concentration. This is in concurrence with our finding that homocysteine concentration was not increased in elderly non-AD patients with depressive disorder. Thus, our data did not support either the additional effect of major depressive disorder on plasma homocysteine concentrations in patients with AD (Chen *et al.* 2010), or significant positive relationship between elevated homocysteine concentrations and currently experiencing depressive symptoms (Forti *et al.* 2010; Gu *et al.* 2012). Although a relation between homocysteine and depressive disorder is biologically plausible, this study indicates no such association in the elderly.

A particularly strong point of this study is the very rigorous diagnosis and clinical evaluation of AD and depressive patients by experienced psychiatrists. The study has some limitations; e.g., the mean age was higher in AD patients compared with controls or patients with depressive disorder. We suppose that adjustment for the age in statistical data analysis is sufficient to correct this discrepancy. Another possible limitation is that important determinants of plasma homocysteine, e.g. creatine, vitamin B_{12} and folate, were not measured in our study. However, when total homocysteine, folate and vitamin B_{12} were measured, the most important finding was the increased level of homocysteine in both AD patients (Morillas-Ruiz *et al.* 2010) and dementia-free elderly subjects (Hooshmand *et al.* 2012). Evidence supports the role of homocysteine as a potential biomarker in age-related neurodegenerative diseases (Herrmann & Obeid 2011); moreover, elevated plasma homocysteine concentrations and low serum folate concentrations are independent predictors of the development of dementia and AD (Ravaglia *et al.* 2005). Thus, we suppose that single plasma homocysteine measurement provides useful information about the AD.

Despite of limitations mentioned above, our work provides important data on association of plasma homocysteine and AD and depression rating scores. The method to assess depression severity was different in the non-AD depressed group and in the AD; however, GDS is considered a reliable alternative in geriatric research comparable to the HRSD (Weintraub *et al.* 2006). Our study can be viewed as a preliminary study that necessitates future work in this area. Most of AD patients were in early stage of the illness. We suppose that some of AD patients with current mild dementia will be repeatedly tested in future to obtain data for longitudinal study of both homocysteine levels and several other potential biomarkers during AD progression.

In conclusion, mean plasma homocysteine concentration was increased in AD compared with controls; the increase was slightly higher in AD without depressive symptoms compared with AD with depressive symptoms as well as in AD with moderate to severe stage of dementia compared with AD with mild stage of dementia. We confirmed that high concentration of plasma homocysteine is associated with increased prevalence of AD and depressive disorder. Significant partial correlation between plasma homocysteine and the MMSE indicate that homocysteine is associated with degree of cognitive impairment in AD and stage of dementia. We did not confirm the positive relationship between homocysteine concentration and the severity of depressive symptoms and we did not find an additive effect of AD and depression on homocysteine plasma concentration. Thus, plasma homocysteine reflects MMSE-assessed degree of cognitive impairment in AD rather than the severity of co-morbid depression. Significantly increased relative risk of disease in people with high plasma homocysteine was found in the AD patients, in subgroups of AD patients with or without depressive symptoms, and in the subgroup of AD patients with moderate to severe dementia. We suppose that high plasma homocysteine may be important component of a panel of biochemical markers of the AD.

ACKNOWLEDGEMENTS

This research was supported by Project MSM0021620849 given by the Ministry of Education, Youth and Sports, by project PRVOUK-P26/LF1/4 given by Charles University in Prague, and by Research Project RVO-VFN64165/2012 given by the General University Hospital in Prague, Czech Republic.

REFERENCES

1 Alexopoulos GS, Meyers BS, Young RC, Campbell S, Silbersweig D, Charlson M (1997). 'Vascular depression' hypothesis. Arch Gen Psychiatry. **54**: 915–922.
2 Almeida OP, McCaul K, Hankey GJ, Norman P, Jamrozik K, Flicker L (2008). Homocysteine and depression in later life. Arch Gen Psychiatry. **65**: 1286–1294.
3 Bicikova M, Hampl R, Hill M, Ripova D, Mohr P, Putz Z (2011). Neuro- and immunomodulatory steroids and other biochemical markers in drug-naive schizophrenia patients and the effect of treatment with atypical antipsychotics. Neuro Endocrinol Lett. **32**: 141–147.
4 Bottiglieri T, Laundy M, Crellin R, Toone BK, Carney MW, Reynolds EH. Homocysteine, folate, methylation, and monoamine metabolism in depression (2000). J Neurol Neurosurg Psychiatry **69**: 228–232.
5 Bottiglieri T. Homocysteine and folate metabolism in depression (2005). Prog Neuropsychopharmacol Biol Psychiatry. **29**: 1103–1112.
6 Cankurtaran M, Yesil Y, Kuyumcu ME, Oztürk ZA, Yavuz BB, Halil M, *et al.* (2013). Altered levels of homocysteine and serum natural antioxidants links oxidative damage to Alzheimer's disease. J Alzheimers Dis. **33**: 1051–1088.
7 Carney MW, Chary TK, Laundy M, Bottiglieri T, Chanarin I, Reynolds EH, *et al.* (1990). Red cell folate concentrations in psychiatric patients. J Affect Disord. **19**: 207–213.

8 Chen CS, Chou MC, Yeh YC, Yang YH, Lai CL, Yen CF, *et al.* (2010). Plasma homocysteine levels and major depressive disorders in Alzheimer disease. Am J Geriatr Psychiatry. **18**: 1045–1048.
9 Connelly PJ, Prentice NP, Cousland G, Bonham J (2008). A randomised double-blind placebo-controlled trial of folic acid supplementation of cholinesterase inhibitors in Alzheimer's disease. Int J Geriatr Psychiatry. **23**: 155–160.
10 de Jager CA, Oulhaj A, Jacoby R, Refsum H, Smith AD (2012). Cognitive and clinical outcomes of homocysteine-lowering B-vitamin treatment in mild cognitive impairment: a randomized controlled trial. Int J Geriatr Psychiatry. **27**: 592–600.
11 Fava M, Mischoulon D (2009). Folate in depression: efficacy, safety, differences in formulations, and clinical issues. J Clin Psychiatry. **70**: 12–17.
12 Folstein M, Liu T, Peter I, Buell J, Arsenault L, Scott T, *et al.* (2007). The homocysteine hypothesis of depression. Am J Psychiatry. **164**: 861–867.
13 Forti P, Rietti E, Pisacane N, Olivelli V, Dalmonte E, Mecocci P, *et al.* (2010). Blood homocysteine and risk of depression in the elderly. Arch Gerontol Geriatr. **51**: 21–25.
14 Gilbody S, Lightfoot T, Sheldon T (2007). Is low folate a risk factor for depression? A meta-analysis and exploration of heterogeneity. J Epidemiol Community Health. **61**: 631–637.
15 Gu P, Defina LF, Leonard D, John S, Weiner MF, Brown ES (2012). Relationship between serum homocysteine levels and depressive symptoms: the Cooper Center Longitudinal Study. J Clin Psychiatry. **73**: 691–695.
16 Herrmann W, Obeid R (2011). Homocysteine: a biomarker in neurodegenerative diseases. Clin Chem Lab Med. **49**: 435–441.
17 Heun R, Kockler M, Ptok U. Depression in Alzheimer's disease: is there a temporal relationship between the onset of depression and the onset of dementia? Eur Psychiatry. 2002;**17**: 254–258.
18 Heun R, Schoepf D, Potluri R, Natalwala A (2013). Alzheimer's disease and co-morbidity: increased prevalence and possible risk factors of excess mortality in a naturalistic 7-year follow-up. Eur Psychiatry. **28**: 40–48.
19 Hooshmand B, Solomon A, Kåreholt I, Leiviskä J, Rusanen M, Ahtiluoto S, *et al.* (2010). Homocysteine and holotranscobalamin and the risk of Alzheimer disease: a longitudinal study. Neurology. **75**: 1408–1414.
20 Hooshmand B, Solomon A, Kåreholt I, Rusanen M, Hänninen T, Leiviskä J, *et al.* (2012). Associations between serum homocysteine, holotranscobalamin, folate and cognition in the elderly: a longitudinal study. J Intern Med. **271**: 204–212.
21 Hroudová J, Fišar Z (2011). Connectivity between mitochondrial functions and psychiatric disorders. Psychiatry Clin Neurosci. **65**: 130–141.
22 Kale A, Naphade N, Sapkale S, Kamaraju M, Pillai A, Joshi S, *et al.* (2010). Reduced folic acid, vitamin B12 and docosahexaenoic acid and increased homocysteine and cortisol in never-medicated schizophrenia patients: implications for altered one-carbon metabolism. Psychiatry Res. 175: 47–53.
23 Lazarou C, Kapsou M (2010). The role of folic acid in prevention and treatment of depression: an overview of existing evidence and implications for practice. Complement Ther Clin Pract. **16**: 161–166.
24 Lipton SA, Kim W-K, Choi YB, Kumar S, D'Emilia DM, Rayudu PV, *et al.* (1997). Neurotoxicity associated with dual actions of homocysteine at the N-methyl-D-aspartate receptor. Proc Natl Acad Sci U S A. **94**: 5923–5928.
25 Lyketsos CG, Olin J (2002). Depression in Alzheimer's disease: overview and treatment. Biol Psychiatry. **52**: 243–252.
26 Lyketsos CG, Carrillo MC, Ryan JM, Khachaturian AS, Trzepacz P, Amatniek J, *et al.* (2011). Neuropsychiatric symptoms in Alzheimer's disease. Alzheimers Dement. **7**: 532–539.
27 Mattson MP, Shea TB (2003). Folate and homocysteine metabolism in neural plasticity and neurodegenerative disorders. Trends Neurosci. **26**: 137–146.
28 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984). Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology. **34**: 939–944.
29 Miller AL (2008). The methylation, neurotransmitter, and antioxidant connections between folate and depression. Altern Med Rev. **13**: 216–226.
30 Mitchell AJ, Bird V, Rizzo M, Meader N (2010). Diagnostic validity and added value of the Geriatric Depression Scale for depression in primary care: a meta-analysis of GDS30 and GDS15. J Affect Disord. **125**: 10–17.
31 Morillas-Ruiz JM, Rubio-Perez JM, Albaladejo MD, Zafrilla P, Parra S, Vidal-Guevara ML (2010). Effect of an antioxidant drink on homocysteine levels in Alzheimer's patients. J Neurol Sci. **299**: 175–178.
32 Mossaheb N, Zehetmayer S, Jungwirth S, Weissgram S, Rainer M, Tragl KH, *et al.* (2012). Are specific symptoms of depression predictive of Alzheimer's dementia? J Clin Psychiatry. **73**: 1009–1015.
33 Nabi H, Bochud M, Glaus J, Lasserre AM, Waeber G, Vollenweider P, *et al.* (2013). Association of serum homocysteine with major depressive disorder: results from a large population-based study. Psychoneuroendocrinology. **38**: 2309–2318.
34 Obeid R, Herrmann W (2006). Mechanisms of homocysteine neurotoxicity in neurodegenerative diseases with special reference to dementia. FEBS Lett. **580**: 2994–3005.
35 Ravaglia G, Forti P, Maioli F, Martelli M, Servadei L, Brunetti N, *et al.* (2005). Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am J Clin Nutr. **82**: 636–643.
36 Refsum H, Nurk E, Smith AD, Ueland PM, Gjesdal CG, Bjelland I, *et al.* (2006). The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. J Nutr. **136**: 1731S–1740S.
37 Seshadri S, Beiser A, Selhub J, Jacques PF, Rosenberg IH, D'Agostino RB, *et al.* (2002). Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N Engl J Med. **346**: 476–483.
38 Seshadri S (2006). Elevated plasma homocysteine levels: risk factor or risk marker for the development of dementia and Alzheimer's disease? J Alzheimers Dis. **9**: 393–398.
39 Smith AD, Smith SM, de Jager CA, Whitbread P, Johnston C, Agacinski G, *et al.* (2010). Homocysteine-lowering by B vitamins slows the rate of accelerated brain atrophy in mild cognitive impairment: a randomized controlled trial. PLoS One. **5**:e12244.
40 Spalletta G, Caltagirone C, Girardi P, Gianni W, Casini AR, Palmer K (2012). The role of persistent and incident major depression on rate of cognitive deterioration in newly diagnosed Alzheimer's disease patients. Psychiatry Res. **198**: 263–268.
41 Starkstein SE, Jorge R, Mizrahi R, Robinson RG (2005). The construct of minor and major depression in Alzheimer's disease. Am J Psychiatry. **162**: 2086–2093.
42 Tiemeier H, van Tuijl HR, Hofman A, Meijer J, Kiliaan AJ, Breteler MM (2002). Vitamin B12, folate, and homocysteine in depression: the Rotterdam Study. Am J Psychiatry. **159**: 2099–2101.
43 Tolmunen T, Hintikka J, Voutilainen S, Ruusunen A, Alfthan G, Nyyssönen K, *et al.* (2004). Association between depressive symptoms and serum concentrations of homocysteine in men: a population study. Am J Clin Nutr. **80**: 1574–1578.
44 Tsolaki M, Papaliagkas V, Kounti F, Messini C, Boziki M, Anogianakis G, *et al.* (2010). Severely stressful events and dementia: a study of an elderly Greek demented population. Psychiatry Res. **176**: 51–54.
45 Van Dam F, Van Gool WA (2009). Hyperhomocysteinemia and Alzheimer's disease: A systematic review. Arch Gerontol Geriatr. **48**: 425–430.
46 Weintraub D, Oehlberg KA, Katz IR, Stern MB (2006). Test characteristics of the 15-item geriatric depression scale and Hamilton depression rating scale in Parkinson disease. Am J Geriatr Psychiatry. **14**: 169–715.
47 Yesavage JA, Brink TL, Rose TL, Lum O, Huang V, Adey M, *et al.* (1982–1983). Development and validation of a geriatric depression screening scale: a preliminary report. J Psychiatry Res. **17**: 37–49.
48 Zeman M, Jirak R, Vecka M, Raboch J, Zak A (2012). N-3 polyunsaturated fatty acids in psychiatric diseases: mechanisms and clinical data. Neuro Endocrinol Lett. **33**: 736–748.
49 Zhuo J-M, Wang H, Praticò D (2011). Is hyperhomocysteinemia an Alzheimer's disease (AD) risk factor, an AD marker, or neither? Trends Pharmacol Sci. **32**: 562–571.