

Serum homocysteine levels in patients with probable vascular dementia

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ABSTRACT

Aim To investigate total homocysteine (tHcy) serum concentration in patients with probable vascular dementia (VD) and in age-matched controls, as well as to determine an association between tHcy serum concentration and cognitive impairment in patients with probable VD.

Methods Serum concentration of tHcy was determined by the Fluorescence Polarization Immunoassay on the AxSYM System. Cognitive impairment was tested by the Mini Mental Status Examination (MMSE) score. Body mass index (BMI) was calculated for each subject included in the study.

Results Age, systolic, diastolic blood pressure and BMI did not differ significantly between the two groups. Mean serum tHcy concentration in the control group of subjects was 13.35 $\mu\text{mol/L}$, while in patients with probable VD it was significantly higher, 19.45 $\mu\text{mol/L}$ ($p=0.002$). A negative but insignificant association between serum tHcy concentration and cognitive impairment in patients with probable VD was found.

Conclusion Increased tHcy concentration in patients with probable VD suggests the possible independent role of Hcy in the pathogenesis of VD.

Key words: neurotoxicity, cognitive impairment, Mini-Mental State Examination

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INTRODUCTION

Vascular dementia represents a clinical syndrome that includes a wide spectrum of cognitive dysfunctions resulting from brain tissue death due to ischemia caused by vascular disease (1). Homocysteine (Hcy) is a thiol-containing amino acid involved in the cycle of methionine as the demethylation product of methionine and in the transsulfuration pathway (1,2). Hyperhomocysteinemia, or increased serum concentration of total homocysteine (tHcy) is a risk factor for cardiovascular disease and seems to be an independent risk factor for dementia (1,2).

Hyperhomocysteinemia is associated with relative deficiencies of folate, vitamins B6 and B12, as well as with older age, male sex, estrogen deficit, renal insufficiency and also with use of different substances like caffeine, dopamine or anticonvulsant drugs (2).

Elevated homocysteine levels can increase oxidative stress and cause endothelial cell dysfunction, smooth muscle cell proliferation and impaired fibrinolysis (2,3). While this could be a contributory factor, data from several incidence studies show that levels in normal elderly people prognosticate the occurrence of stroke and dementia (2-4). Moreover, it is possible that deficiency of vitamin B12 or folic acid could result in metabolic derangements other than hyperhomocysteinemia (2-4). Thus, hyperhomocysteinemia should, at the present time, be regarded only as an indicator of susceptibility to disease, and therapies that could reduce Hcy levels are not guaranteed to reduce the risk and incidence of either strokes or dementia (5).

Homocysteine promotes changes associated with atherosclerosis but the pathological mechanisms triggered by Hcy accumulation are poorly understood (6). The effect of atherosclerosis on dementia has also been attributed to its relation to cerebral infarction or to systemic or local factors that underlie both atherosclerosis and cognition (7).

Several studies have shown significantly higher serum Hcy concentration in patients with VD compared to control subjects (8). Studies have shown a correlation between low folic acid and high Hcy levels with cognitive functions in vascular dementia and AD, which is consistent with the idea of secondary elevation of total Hcy in demented people. (9).

The aim of this study was to investigate serum tHcy concentration in patients with probable VD

with age-matched controls. We also aimed to determine whether there is an association between serum tHcy concentrations and cognitive impairment tested by Mini Mental Status Examination (MMSE) score at patients with probable VD.

PATIENTS AND METHODS

Patients and study design

The study was designed as a cross-sectional study which included 20 patients (17 females and 3 males) with probable VD aged 65 years old and more, institutionalized at the specialized unit at the Health-Care Hospice for persons with disabilities in Sarajevo, Bosnia and Herzegovina. Furthermore, in this study 19 community-dwelling, age-matched apparently healthy controls without dementia were included.

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

An approval for the study was obtained by the Ethics Committee of Clinical Centre, University of Sarajevo. All procedures on human subjects were performed according to the Declaration of Helsinki, 1975. Written informed consent was obtained from all participating subjects and caregivers.

Methods

Probable vascular dementia was clinically diagnosed by standardized clinical examination conducted by a specialist neurologist and psychiatrist by the NINDS-AIREN criteria (10).

Global cognitive function was tested with the MMSE test which has been used for rapid screening of those with cognitive and/or intellectual deficit (11). The test evaluates orientation, short term memory, serial subtraction, constructional capacities and use of language. The total score was 30, where the score of 24 was considered abnormal, and the score less than 17 was considered as dementia. All patients with probable VD had a score ≤ 12 while subjects in the control group had a score from 26 to 30.

The Hachinski ischemic score (HIS) differentiates patients with VD from those with AD (12). The original scale consists of 13 items where each scale item represents a specific clinical event which has a certain numeric value. A score

less or equal 4 means Alzheimer's dementia, 4-7 indicates mixed dementia, and a total score of ≥ 7 refers to vascular dementia. Patients in this study with probable VD had a score ≥ 7 .

Non-fasting blood samples were drawn from the antecubital vein into siliconized tubes. After venipuncture, blood samples were put on ice. The median time between venipuncture and centrifugation was 50 min (interquartile range: 30-70 min). Serum samples were stored at or below -20°C .

Serum tHcy concentration was measured by using a fluorescence polarization immunoassay on the AxSYM System at the Institute for Chemistry and Biochemistry, Clinical Centre of the University of Sarajevo (13). The reference interval for tHcy concentration with the use of this method was from 3.36 to 20.44 $\mu\text{mol/L}$.

Body Mass Index (BMI) for all participants was calculated as weight (kg) divided by the square of height in meters (m^2). Height was measured with stadiometer and body weight was measured with the use of Toledo self-zeroing weight scale.

Trained staff measured blood pressure using a mercury sphygmomanometer on the right arm after a 5 minute rest.

Statistical analysis

Data were presented as mean \pm SEM. Data distribution was determined using the Shapiro-Wilk test. Data were statistically analyzed by using Student's t test. Additionally, Pearson correlations were used as measures of association for the continuous variables. Statistical significance was set at $p < 0.05$.

RESULTS

No differences emerged in age, systolic and diastolic blood pressure between the groups. No difference in BMI was found between the two groups. Subjects with probable VD had statistically significantly lower MMSE scores compared with the control group ($p < 0.001$) (Table 1).

Mean serum tHcy concentration in the control group was 13.35 $\mu\text{mol/L}$, while in patients with probable VD the mean serum tHcy concentration was significantly higher, 19.45 $\mu\text{mol/L}$ ($p = 0.002$) (Figure 1).

A negative but insignificant correlation was noted between tHcy concentration and MMSE score in patients with probable VD ($r = -0.05$).

Table 1. Baseline characteristics of patients with probable vascular dementia and the control group*

Variables	Patients with probable vascular dementia	Controls
Age (years)	76,9 \pm 1,4	77,8 \pm 1,1
SBP (mmHg)	132,0 \pm 6,6	130,5 \pm 4,4
DBP (mmHg)	80,0 \pm 3,4	81,3 \pm 1,9
BMI (kg/m^2)	23.9 \pm 0,5	25,6 \pm 0,8
MMSE score	8,0 \pm 1,3 \dagger	27,6 \pm 0,3

*Data are presented as mean \pm S.E.M.; $\dagger p < 0.001$; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; MMSE, Mini Mental State Examination;

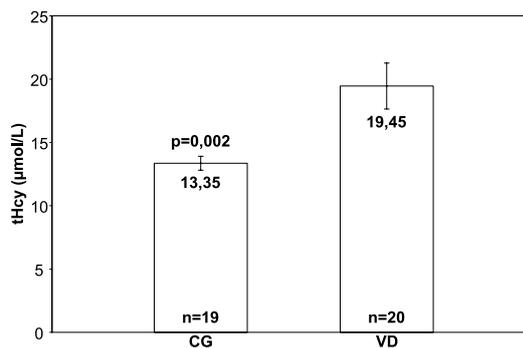


Figure 1. Mean serum total homocysteine concentration (tHcy) in the control group (CG) and in patients with probable vascular dementia (VD)

Data are presented as mean \pm S.E.M.

DISCUSSION

Previous cross-sectional studies have found elevated Hcy levels in patients with VD (8,14,15). It is believed that there are several mechanisms between increased Hcy levels and the occurrence of VD. One of them explains that homocysteine has a direct neurotoxic effect which play a significant role in the etiology of VD and is also associated with cerebrovascular disease (14). More recently, observational studies have begun to link high concentrations of homocysteine to Alzheimer's disease and other dementias (15).

The main finding of this study is a significantly higher serum tHcy concentration in patients with probable VD compared with the control group. Our observations are consistent with those of Malaguarnera et al. who also found elevated serum concentration levels of Hcy in patients with VD compared with the control group of subjects (8). Follin et al. showed a significant increase in plasma Hcy levels in patients with AD and VD compared with controls. These results showed that the VD group had the highest levels and also elevated Hcy levels showed a great involvement in dementia (16). Several studies also reported

that persons with elevated serum homocysteine concentrations have a significantly greater risk of developing dementia than the persons without elevated homocysteine (17,18), but the results have not been entirely consistent (19). It is unclear whether low concentrations of vitamin B-12 or folate acid were responsible for the greater risk of dementia or elevated serum homocysteine concentrations in those studies. Haan et al. reported significant associations of dementia or cognitive impairment with elevated plasma homocysteine concentrations in a 4.5-year follow-up of a cohort study, conducted after the introduction of mandatory folic acid fortification in the United States of 1779 Mexican Americans aged 60 years (20). While there is a strong correlation between hyperhomocysteinemia on one side and cerebrovascular disease and dementia on the other, a correlation should never be taken to indicate causation (2,3,21). Homocysteine itself is thought to be toxic to blood vessels and can be shown in vitro to cause excitotoxic damage like high levels of glutamate (2,3,21). Oxidative stress can be one of the mechanism involved in endothelial dysfunction. Therefore, oxidative stress could affect endothelial cells in large vessels (e.g. the carotid arteries) or smaller-caliber arterioles (such as penetrating blood vessels in the brain) resulting in local thrombosis and cerebral ischemia (2,3,21).

Kloppenborg et al. (22) found that patients with symptomatic atherosclerotic disease and higher homocysteine levels are associated with higher presence of lacunar infarcts and slightly worse cognitive function. The same statement about high level of plasma homocysteine as an independent risk factor for cerebral large-artery atherosclerosis was also reported by Wang et al. (23). Alternatively, large vessel intracranial atherosclerosis could be a marker for dysfunction of

small cerebral vessels and their endothelium that might be the proximate cause of cognitive deterioration, either through disruption of the communication between neurons and blood vessels (the neurovascular unit) that underlies activity induced vasodilatation, or through disruption of the blood-brain barrier (7).

A 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 VD or mixed AD/VD showed increased tHcy. Total Hcy was also elevated in patients with LOAD and a history of cerebrovascular disease (CVD) compared with both AD patients without history of CVD and with controls. These findings suggest that elevated tHcy contributes to dementia mainly through vascular mechanisms (24).

When interpreting our results a number of limitations should be considered. A limitation of the study was a small sample size. As we analyzed cross sectional data our study did not demonstrate a causal relationship. Increased Hcy concentration in patients with probable VD observed in our study suggest a possible role for Hcy in the pathogenesis of this disease. These findings imply that the serum Hcy level may be a potential biomarker in the diagnosis of vascular dementia. Further studies are needed to establish that elevated homocysteine is a risk or a consequence of VD as well as evaluate the potential biomarkers role of homocysteine in this disease.

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TRANSPARENCY DECLARATIONS

Competing interests: none to declare.

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Nivo homocisteina u serumu pacijenata s mogućom vaskularnom demencijom

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SAŽETAK

Cilj Ispitati koncentraciju ukupnog homocisteina (tHcy) u serumu pacijenata s mogućom vaskularnom demencijom (VD) i kod ispitanika kontrolne grupe, te utvrditi povezanost između nivoa tHcy i kognitivnog oštećenja kod pacijenata s mogućom VD-om.

Metode Serumaska koncentracija tHcy određivana je metodom fluorescentno-polarizacijskog imunoseja na AxSYM sistemu. Kognitivno oštećenje je testirano skorom minimalnog statusa (MMSE). Indeks tjelesne mase (ITM) izračunat je za svakog ispitanika uključenog u studiju.

Rezultati Starosna dob, sistolni i dijastolni krvni pritisak i ITM nisu se značajno razlikovale između dvije ispitivane grupe. Prosječna koncentracija tHcy u kontrolnoj grupi iznosila je 13.35 μmol/L, dok je u grupi pacijenata s mogućim VD-om bila signifikantno viša i iznosila je 19.45 μmol/L (p=0.002). Utvrđena je negativna, ali ne signifikantna povezanost između serumske koncentracije tHcy i kognitivnog oštećenja kod pacijenata s mogućim VD-om.

Zaključak Povećan nivo tHcy kod pacijenata s mogućim VD-om sugeriše na moguću nezavisnu ulogu Hcy u patogenezi VD-a.

Ključne riječi: neurotoksičnost, kognitivno oštećenje, minimalni status