Research Journal of Biological Sciences 3 (11): 1337-1340, 2008
ISSN: 1815-8846

Hyperhomocysteinemia in Patients with Cognitive Impairment

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Abstract: Cognitive impairment is common in elderly people and represents clinical feature of neurodegenerative diseases. Not all of patients with Mild Cognitive Impairment (MCI) finally develop dementia and it is interesting to investigate the role of possible markers for early diagnosis. Hyperhomocysteinemia is associated to several pathologies including cognitive impairment; aim of this study is to evaluate the correlation between cognitive performance assessment and homocysteine plasma levels. Total 74 patients and 75 healthy controls were enrolled and MCI were defined by a MMSE score lower than 26 after adjustment for years of schooling. Homocysteine plasma levels were determined. Homocysteine levels significantly raised in patients with cognitive impairment and showed a significant negative association with MMSE score. Finally, our data show that a moderate risk of cognitive impairment could be associated to high homocysteine plasma levels.

Key words: Cognitive impairment, homocysteine, B_{12} vitamin, folate

INTRODUCTION

Cognitive impairment represents a clinical feature of neurodegenerative diseases leading to dysfunction and finally death of subsets of neurons. However, Alzheimer's Disease (AD), Vascular Dementia (VD) and others are condition that are at the severe end of the spectrum of cognitive impairment in elderly subjects. A wider range, from mild to moderate, of cognitive impairment is common and could be defined by memory impairment, while general cognitive function and daily living activities are preserved. Furthermore, a nosological entity considered at risk for developing AD is represented from Mild Cognitive Impairment (MCI) (Jelic *et al*., 2007), this term refers to a transitional state between normal aging and Alzheimer Dementia; nevertheless, some MCI patients do not develop dementia and it led the interest in the role of possible markers for early diagnosis. Hyperomocysteinemia is considered a marker of low B_{12} vitamin and folate levels (Prins *et al*., 2002; Ravaglia *et al*., 2005; Seshadri and Wolf, 2002); low B_{12} and folate and raised homocysteine have been linked to a greater risk of Alzheimer disease and this has led to interest in the role of folate and B_{12} dietary intake in the prevention and management of cognitive impairment; while the role of vitamin supplementation is controversial, the relationship between serum B_{12} and folate and cognitive function have been reported in several studies (Budge *et al*., 2000; Clarke *et al*., 1998; Quadri *et al*., 2004); in a prospective 8-years study performed on dementia-free subjects from the 20th examination of Framingham Study homocysteine has been considered as independent marker of developing AD (Elias *et al*., 2005).

Aim of this study is to evaluate the differences in B_{12} vitamins, folate and homocysteine serum concentrations between elderly patients with a mild cognitive impairment and those without and verify the association between those biochemical markers and the subtype of cognitive impairment (vascular vs neurodegenerative).

METERIALS AND METHODS

Patients: We enrolled 74 patients (40 males, 34 females) referred to the Unit of Clinical Chemistry for serum B vitamin and homocysteine assessment; blood samples were sent from the Department of Internal Medicine and taken from patients admitted from September 2005 to September 2007 for a clinical evaluation of their internistic and cardiovascular diseases (with the exclusion of acute cardiovascular and cerebrovascular events). Seventy five age-matched healthy controls were recruited for determining baseline characteristics and

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laboratory analysis. During hospital stay, all subjects underwent a complete anamnesis and objective examination, in addition to biochemical analysis; cognitive function and disability assessment were determined with Mini-mental state examination and ADL score(Katz, 1963), respectively. All cases (patients with MCI) were defined by a MMSE score lower than 26 after adjustment for years of schooling and no disability evaluated with ADL score. Exclusion criteria were B vitamins supplementation therapy and BMI <20. Hachinsky Ischaemia Score, a tool used in screening to differentiate vascular dementia from degenerative forms of the disorder, was administered only to subjects with a MMSE score <26, adjusted for age and years of schooling (Crum *et al*., 1993); Hachinsky Ischaemia Score of 7 or higher was considered more likely to suggest a vascular dementia, while 4 or lower show possible underlying neurodegenerative disease (Monorey *et al*., 1997).

Laboratory measurement: For laboratory measurement fasting blood samples were taken in all subjects before testing cognitive function; pre-analytical procedures were executed according to photosensitivity characteristics of B_{12} vitamin and folate. B vitamins and homocysteine serum concentrations were determined by a solid-phase, two-site, chemiluminescent enzyme immunometric assay (Immulite; Medical Systems, Italy). Homocysteinemia is expressed in μmol/L.

Statistical analysis: Baseline differences between cases and control subjects were examined by means of the χ^2-test for categoric data and an unpaired Student's t-test for continuous data. Correlations between MMSE scores and serum homocysteine, B_{12} and folate levels were estimated by using Spearman's distribution-free method, while the association between subtype of cognitive impairment and biochemical markers levels (Hcy, B_{12} and folate) was examined with the use of a logistic regression model after adjustment for age, sex and conventional vascular risk factors (hypertension, diabetes, hypercholesterolemia, current smoker, history of previous vascular events). Results were expressed as odds ratios, together with their 95% CI.

RESULTS

As shown in Table 1, subjects with MCI have a significant higher rate of hypertension, smoking habit, proteinuria/microalbuminuria, previous cardio- and cerebrovascular events and chronic renal failure; moreover, as expected, they are treated with statins or antiplatelet drugs more than controls. For cases, Hachinsky Ischaemia Score was higher than 7 in 37 (50%) subjects and lower than 4 in 25 (33.8%). Serum total homocysteine levels were significantly raised ($p<0.05$) and B_{12} and folate were significantly decreased among cases ($p<0.05$) (Table 2). Homocysteine showed a significant negative association with MMSE score among case (Table 3). The multivariate analysis, after adjustment for age, diabetes, hypertension, history of cardiovascular or cerebrovascular events and for creatinine serum concentrations showed a significant association between a neurodegenerative kind of MCI and homocysteine levels (Table 4).

Table 1: Clinical and baseline laboratory variables in patients with and without mild cognitive impairment

	Patients with MCI (74)	Patients without MCI (75)	p-value
Sex: men/women (n%)	40/34 (54.4/45.9)	40/35 (53.3/46.7)	n.s.
Age (yrs)	71.7±4.8	71.3±4	n.s.
Diabetes (n%)	24 (32.2)	23 (30.6)	n.s.
Hypertension (n%)	47 (63.5)	36 (48%)	<0.05
Total Cholesterolemia (mg dL^{-1})	227.5±42.1	216.6±42.2	n.s.
LDL Cholesterolemia (mg dL^{-1})	140±10.8	128.6±11.6	<0.05
HDL Cholesterolemia (mg dL^{-1})	37.6±17.3	42.4±19.9	n.s.
Triglyceridemia (mg dL^{-1})	180.2±57.7	149.8±63.5	n.s.
Serum Uric Acid (mg dL^{-1})	5.6±1.6	4.4±2.2	n.s.
BMI (kg m^{-2})	31.7±6.2	31.0±4.5	n.s.
Smoking (n%)	28 (37.8)	22 (29.8)	<0.05
Cholesterol plasma levels >200 mg dL^{-1} (n%)	21 (28.3)	23 (30.06)	n.s.
Triglyceride plasma levels >150 mg dL^{-1} (n%)	22 (29.7)	18 (24)	n.s.
LDL plasma levels >130 g dL^{-1} (n%)	20 (27.02)	20 (26.6)	n.s.
Microalbuminuria/Proteinuria (n%)	64 (62.7)	21 (28)	<0.05
Previous cardiovascular events (n%)	41 (55.5)	24 (32)	<0.05
Previous cerebrovascular events (n%)	33 (44.5)	20 (26.6)	<0.05
Renal failure (n%)	16 (21.6)	9 (12)	<0.05
Statin therapy (n%)	39 (52.3)	30 (40)	<0.05
Antiplatelet therapy (n%)	58 (78.3)	23 (30.6)	<0.05
MMSE score (median)	22	27	
Hachinsky Ischaemia Score of ≥7 (n%)	37 (50)		
Hachinsky Ischaemia Score of ≤4 (n%)	25 (33.8)		

Laboratory values are represented as means±SD and tested with unpaired t-student test; demographic variables are expressed as number/percentage and tested with Chi-square test

Table 2: Metabolic variables in patients with and without MCI

	Cases (74)	Controls (75)	p-value
Homocysteine (μmol L^{-1})	36.72±12.48	12.6±5.43	<0.001
B_{12} vitamin (pg mL^{-1})	96±29	204±80	<0.05
Folate (ng mL^{-1})	3.3±0.6	4.8±0.9	<0.05

Values are represented as means±SD and tested with unpaired t-student test

Table 3: Correlations between MMSE scores and serum homocysteine, vitamin B_{12} and folate levels Spearman's rho

	MMSE in cases (74)	MMSE in controls (75)
Homocysteine	-0.211*	-0.031
B_{12} vitamin	0.042	0.026
Folate	0.07	-0.07

*p<0.05

Table 4: Adjusted multivariate analysis of relationship between homocysteine, vitamin B_{12} and folate plasma levels and type of MCI (Adjustment for age, sex and conventional vascular risk factors)

Patients with MCI (74)	H.I. score <4 (n 25)	H.I. score >7 (n 37)
	HR (95% CI)	HR (95% CI)
Homocysteine	1.79 (1.3-2,.1)	0.95 (0.45-1.2)
Vitamin B_{12}	0.95 (0.35-1.1)	0.54 (0.31-0.87)
Folate	0.71 (0.23-0.89)	0.67 (0.35-1.1)

DISCUSSION

Elevated total homocysteine (tHcy) concentrations have been associated with cognitive impairment, but it is unclear whether low vitamin B-12 or folate status is responsible for cognitive decline (Clarke *et al*., 2007). The Hcy hypothesis of dementia has attracted considerable interest, as Hcy can be easily lowered by folic acid and vitamin B12, raising the prospect that B-vitamin supplementation could lower the risk of dementia (Rowan *et al*., 2007). Hyperhomocysteinaemia has been associated with risk of stroke, Myocardial Infarction (MI), Alzheimer's Disease (AD) and vascular dementia (Miller *et al*., 2002). Studies of cognitive decline and tHcy in healthy controls, however, conflict. Vascular disease patients may have higher tHcy than AD patients, thus, hyperhomocysteinaemia in demented subjects could be due to concomitant vascular disease, rather than a cause of dementia (Clarke, 2008). This, however, is controversial since elevated tHcy has existed in pathologically confirmed AD cases both with and without vascular disease as well as in vascular dementia. Regarding on B vitamin role in conditioning cognitive performance some authors (Vidal *et al*., 2008), reported an association between high homocysteinemia and decreased cognition only seen in patients with low folate levels, suggesting that folate may influence the course of cognitive decline. Although, high homocysteine concentrations are associated with poorer cognitive function it is known that hyperhomocysteinemia can be influenced by a number of factors including age, sex and diet. However, is still under investigation (Schulz, 2007), whether reduction of hyperhomocystemia is reducing the risk of dementia or Alzheimer's disease. Furthermore, it is still evaluated the association between dementia and common vascular risk factors certainly including homocysteine; as suggested by some Authors (Elkins *et al*., 2007), who performed a prospective study in a population of very old people it seems that the contribution of vascular risk factors to the risk of dementia may be age-dependent and their role in the very old subjects may be mediated through their influence on cerebrovascular morbidity. Our findings are consistent with literature recent data assessing an association between cognitive impairment and both high plasma homocysteine levels and low serum folate concentration (Clarke *et al*., 1998; Quadri *et al*., 2004; Seshadri and Wolf, 2002). According to Quadri *et al*. (2004) and Clarke *et al*. (1998), significant differences in those metabolite concentrations between case and controls. The reported negative association of hyperho-mocysteinemia and MMSE score is consistent with prevous studies (Seshadri *et al*., 2002), while the Rotterdam study didn't find this association (Kalmijn *et al*., 1999). The association between hyperhomocy-steinemia and neurodegenerative subtype of cognitive impairment is controversial but consistent with the work of Hogervost *et al*. (2002) that suggest a possible direct neurotoxic effect and a role of independent risk factor for leukoaraiosis and AD respectively (Seshadri and Wolf, 2003). Finally, our data are consistent with Sacramento Area Latino Study (Ramos *et al*., 2005) showing a consistent risk of cognitive impairment associated to high homocysteine levels. Serum homocysteine increases with age and can be influenced by life styles (smoking habit), so a larger sample study should be considered. Serum folate levels showed a mild negative but not significant association among cases with neurodegenerative subtype of cognitive impairment and this could be explained with a relative small sample size in our population.

ACKNOWLEDGEMENT

The authors acknowledge Dr. Egidio Guglielmini for his technical analysis.

REFERENCES

Budge, M., C. Johnston and E. Hogervorst *et al*., 2000. Plasma total homocysteine and cognitive performance in a volunteer elderly population. Ann. N.Y. Acad. Sci., 903: 407-410.

Clarke, R., 2008. B-vitamins and prevention of dementia. Proc. Nutr. Soc., 67 (1): 75-81.

Clarke, R., J. Birks, E. Nexo, P.M. Ueland, J. Schneede, J. Scott, A. Molloy and J.G. Evans, 2007. Low vitamin B-12 status and risk of cognitive decline in older adults. Am. J. Clin. Nutr., 86 (5): 1384-1391.

Clarke, R., A.D. Smith, K.A. Jobst, H. Refsum, L. Sutton and P.M. Ueland, 1998. Folate, vitamin B12 and serum total homocysteine levels in confirmed Alzheimer disease. Arch. Neurol., 55: 1449-1455.

Crum, R.M., J.C. Anthony, S.S. Bassett and M.F. Folstein, 1993. Population-based norms for the Mini-Mental State Examination by age and educational level. JAMA., 269: 2386- 2391.

Elias, M.F., L.M. Sullivan and R.B. D'Agostino *et al*., 2005. Homocysteine and cognitive performance in the Framingham offspring study: Age is important. Am. J. Epidemiol., 162: 644-653.

Elkins, J.S., S.C. Johnston, E. Ziv, D. Kado, J.A. Cauley and K. Yaffe, 2007. Methylenetetrahydrofolate reductase C677T polymorphism and cognitive function in older women. Am. J. Epidemiol., 166 (6): 672-978.

Hogervorst, E., H.M. Ribeiro, A. Molyneux, M. Budge and A.D. Smith, 2002. Plasma Homocysteine Levels, Cerebrovascular Risk Factors and Cerebral White Matter Changes (*Leukoaraiosis*) in Patients With Alzheimer Disease. Arch. Neurol., 59: 787-793.

Jelic, V., M. Kivipelto and B. Winblad, 2007. Clinical trials in mild cognitive impairment: lessons for the future. J. Neurol. Neur. Psychiatr., 77: 429-438.

Kalmijn, S., L.J. Launer, J. Lindemans, M.L. Bots, A. Hofman and M.M.B. Breteler, 1999. Total Homocysteine and Cognitive Decline in a Community-based Sample of Elderly Subjects: The Rotterdam Study. Am. J. Epidemiol., 150: 283-289.

Katz, S., A.B. Ford, R.W. Moskowitz, B.A. Jackson and M.W. Jaffe, 1963. Studies of illness in the aged. The index of ADL: A standardized measure of biological and psychosocial function. JAMA., 185: 914-919.

Miller, J.W., R. Green, D.M. Mungas, B.R. Reed and W.J. Jagust, 2002. Homocysteine, vitamin B6 and vascular disease in AD patients. Neurology, 58: 1471-1475.

Moroney, J.T., E. Bagiella and D.W. Desmond *et al*., 1997. Meta-analysis of the Hachinski Ischemic Score in pathologically verified dementias. Neurology, 49: 1096-1105.

Prins, N.D., T. Den Heijer and A. Hofman *et al*., 2002. Homocysteine and cognitive function in the elderly: The Rotterdam Scan Study. Neurology, 59: 1375-1380.

Quadri, P., C. Fragiacomo and R. Pezzati *et al*., 2004. Homocysteine, folate and vitamin B- 12 in mild cognitive impairment, Alzheimer disease and vascular dementia. Am. J. Clin. Nutr., 80: 114-122.

Ramos, M.I., L.H. Allen and D.M. Mungas *et al*., 2005. Low folate status is associated with impaired cognitive function and dementia in the Sacramento Area Latino Study on Aging. Am. J. Clin. Nutr., 82: 1346-1352.

Ravaglia, G., P. Forti and F. Maioli *et al*., 2005. Homocysteine and folate as risk factors for dementia and Alzheimer disease. Am. J. Clin. Nutr., 82: 636-643.

Rowan, E.N., H.O. Dickinson, S. Stephens, C. Ballard, R. Kalaria and R.A. Kenny, 2007. Homocysteine and post-stroke cognitive decline. Age Ageing, 36 (3): 339-343.

Seshadri, S., A. Beiser and J. Selhub *et al*., 2002. Plasma homocysteine as a risk factor for dementia and Alzheimer's disease. N. Engl. J. Med., 346: 476-483.

Schulz, R.J., 2007. Homocysteine as a biomarker for cognitive dysfunction in the elderly. Curr. Opin. Clin. Nutr. Metab Care, 10 (6): 718-723.

Seshadri, S. and P.A. Wolf, 2003. Homocysteine and the brain: Vascular risk factor or neurotoxin? Lancet Neurol., 2: 11.

Vidal, J.S., C. Dufouil, V. Ducros and C. Tzourio, 2008. Homocysteine, Folate and Cognition in a Large Community-Based Sample of Elderly People-The 3C Dijon Study. Neuroepidemiology, 30 (4): 207-214.