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Association of Cognitive Impairment with Plasma Levels of Folate, Vitamin B_{12} and Homocysteine in the Elderly

NIKOLAOS DIMOPOULOS[1], CHRISTINA PIPERI[1], ARISTEA SALONICIOTI[2], VASSILIKI PSARRA[3], FLERRI GAZI[3], CHARALAMBOS NOUNOPOULOS[1], ROBERT W. LEA[4] and ANASTASIOS KALOFOUTIS[1]

[1]*Department of Biological Chemistry of Medical School of Athens University 75 M. Asias St., Athens;*
[2]*Health Center, Markopoulo, Mesogia Attica;*
[3]*Psychiatric Hospital of Attica "Dafni" Haidari, 374 Athinon Ave., Greece;*
[4]*Department of Biological Sciences, University of Central Lancashire, Preston, U.K.*

Abstract. *Background: Alterations in folate, vitamin B_{12} and homocysteine plasma levels have been associated with aging, neuronal development and cognitive impairment. The aim of this study was to investigate the relationship between plasma folate, vitamin B_{12} and homocysteine and cognitive function in the elderly. Patients and Methods: Elderly individuals over 60 years of age living in the community, were screened with the Mini-Mental State Examination. The study population was divided into two groups: a) 37 subjects with dementia; and b) 33 healthy controls. Blood samples were analyzed with the use of ELISA, and the results were statistically evaluated at $p<0.05$ level of significance. Results: Group a had significantly lower levels of folate and vitamin B_{12} than group b. Homocysteine was significantly higher in demented individuals than in controls ($p<0.01$). Conclusion: Lower levels of plasma folate and/or vitamin B_{12} and higher levels of plasma homocysteine are associated with cognitive impairment in elderly individuals.*

Elderly individuals represent a rapidly growing segment of the community worldwide. From 1996 to 2025, the percentage of people over 60 years of age is expected to increase by 17 to 82% in European countries, and by 200% in some developing countries. The world's "oldest" countries, with a large percentage of the population over 60, are Italy and Greece (1). As more people are living longer, the large burden of chronic disease-associated morbidity and/or disability is predictive of further illness, injury, hospitalization, institutionalization and death. One of the most common disorders in the elderly is dementia, which contributes substantially to the decline in the quality of life of senior citizens.

The prevalence of dementia rises exponentially with age. Studies in developed countries have established a dementia prevalence of between 1-5% at the age of 65 years, whereas it is approximately 6.5% at 75 (2, 3). Between 80-84 years of age it is estimated as 10.5% (4), while over 85 years of age, prevalence exceeds 20% (5).

Considerable attempts at discovering biological markers for dementia have been made. Such markers may play a key role in the early diagnosis and management of the disorder, as there are now medications which delay the course of dementia, especially in the stage of mild cognitive disorder. However, as yet there is no clinical method to determine if a patient with mild cognitive impairment will develop dementia or when this may occur (6). Thus, there is a clinical need for diagnostic biomarkers to identify incipient dementia and geriatric individuals with cognitive impairment.

Several studies identified associations between low folate status (7) or elevated homocysteine concentrations (8) and cognitive dysfunction. Low folate plasma levels were associated with both Alzheimer's disease (AD) and vascular dementia (7, 9, 10). There are studies that showed a high incidence of folate deficiency correlated with mental symptoms, especially depression and cognitive decline, in geriatric and psychogeriatric populations (11).

Lower plasma vitamin B_{12} levels were observed in individuals with AD, (12) other dementias (13) and in people with different cognitive impairments (12) when compared to controls.

Large epidemiological studies (The Rotterdam Scan Study, The Third National and Nutrition Examination Survey, Sacramento Area Latino Study on Aging) have revealed a positive relationship between cognitive decline and increased plasma homocysteine (HCY) in the elderly

Correspondence to: Nikolaos Dimopoulos, MD, MSc, Laboratory of Biological Chemistry, University of Athens Medical School, M. Asias 75 Goudi 11527, Athens, Greece. e-mail: dmpnikos@yahoo.gr

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(14, 15). However, a number of epidemiological studies in populations of various ethnicities were unable to find any relationship between folate, vitamin B_{12} or homocysteine and cognitive impairment (16-19).

There is a discrepancy for all three factors (folate, B_{12} and HCY) regarding their relationship with dementia and a crucial question is whether observed associations are a cause or consequence of the disease. It may be argued that dementia in the elderly leads to a reduced dietary intake of folate and vitamin B_{12}, causing an elevation in HCY levels (20), and that low folate and vitamin B_{12} levels may be related to their effects on methylation reactions in the brain (21) or may be mediated by their effects on HCY levels (22). HCY may have a neurotoxic effect leading to cell death (23), or it might be converted into homocysteic acid, which also has a toxic effect on neurons (24). In addition, elevated HCY levels are damaging for blood vessels and a strong risk factor for vascular disease (7). The extensive literature and the ongoing efforts to elucidate the role of these factors in neuropsychiatric disorders, in combination with the high prevalence of vitamin deficiency in the elderly and the convenience of their measurement in the peripheral blood, makes them attractive possible candidates as biochemical markers for dementia.

The aim of this present study was to investigate the relationship between plasma folate, vitamin B_{12} and HCV in cognitive dysfunction in the elderly, and the possible diagnostic utility of these parameters, even though they may not be specific markers for this disorder.

Patients and Methods

The study population consisted of Greek community-dwelling, older adults, recruited over a period of 15 months. The main source of individuals was the local municipal centers, where the elderly gather in order to meet and socialize. In addition, recruitment also took place by door-to-door canvassing. Eligible subjects included individuals over 60 years of age, with no history of any relevant psychiatric disease and no systematic use of psychotropic drugs or substance abuse. The study was conducted in accordance with the Declaration of Helsinki, and all procedures were carried out with the adequate understanding and written consent of the subjects or close relatives when necessary.

Initially, all subjects were screened with the Mini-Mental State Examinatiion. Individuals who scored abnormally were further clinically evaluated for dementia. The diagnosis of dementia was made according to the criteria of Diagnostic and Statistical Manual IV. After application of these criteria, the "dementia" group (A) consisted of 37 subjects, who agreed to give a blood sample and complete the study. In parallel, another group of 33 healthy controls (B) was selected from the same population. The participants of the last group scored normally in both rating scales but failed to meet the diagnostic criteria for dementia or depression, although they fulfilled the rest of the inclusion criteria.

Mental scale. The Mini-Mental State Examination, developed by Folstein in 1975, is a screening tool that gives a brief assessment of an individual's orientation to time and place, recalling ability, short memory and arithmetic ability (25). The Mini-Mental State Examination (MMSE) is a valid and reliable method widely used for assessing cognitive mental status in older adults (26). It has 11 items and tests five areas of cognitive function: orientation, registration, attention and calculation, recall and language. It can be scored immediately with a maximum of 30 (no impairment). In detail, scores between 26 and 30 are considered normal in the general population. Patients who score between 20 and 25 have mild cognitive impairment, but usually live on their own support despite the problems of every day living (shopping, financial matters, *etc.*). Scores between 10 and 20 indicate moderate deterioration and disability of independent living and patients who score below 10 have severe cognitive impairment and require support for basic activities, such as eating and walking (27).

Blood sampling. Overnight fasting blood was collected from each participant by standard venepuncture into evacuated tubes with and without EDTA or heparin. Blood samples were centrifuged at 1,500 rpm and plasma and serum isolated and stored at –80˚C until required for analysis.

Determination of plasma folate, vitamin B_{12} and HCY concentrations. Plasma folate, vitamin B_{12} and HCY concentrations were measured by chemiluminescence using the Bayer ADVIA Centaur System (Bayer Corporation, Tarrytown, NY, USA). The sensitivity and the assay ranges for folate, vitamin B_{12} and HCY by this method were 0.8 54.4 nM and 33.2 1476 pM and 4.9-13.9 μM, with interassay variations 2.8%, 3.9% and 3.5%, respectively.

Statistical analysis. Demographic, clinical and laboratory data were collected and statistically analyzed with the use of an SPSS program for Windows. The Pearson Chi-square test was used to compare categorical variables. For quantitative values, data were expressed as mean±standard deviation (SD). Due to the lack of normality of distribution of the values non-parametrical tests were used (Kruskal-Wallis and Mann-Whitney *U*-test). Statistical significance was set at $p<0.05$. Additionally Spearman correlations were used as measures of association for the continuous variables.

Results

The principal demographic characteristics of the two groups are shown in Table I. During statistical analysis, the dementia group as a whole was assessed without taking into consideration the different kinds of dementia. The mean age (±SD) of the dementia and control groups was 69.72±9.50 and 65.39±4.06, respectively. There was no statistically significant difference for the age of individuals in the two groups ($p>0.05$). The female gender was dominant in all groups with a percentage of 59.46% and 60.61%, respectively. The majority of the individuals were married, whereas widows constituted the second most common status; 72.97% and 57.57% of the dementia and control groups, respectively, had a minimum educational level (literate and primary school).

Table I. *The main socio-demographic and medical characteristics of the subjects with or without dimentia (n=70).*

	N(all)	Dementia	Controls	*P*-value*
Gender				$p>0.05$
Male	28	15 (53.5%)	13 (46.5%)	
Female	42	22 (52.3%)	20 (47.7%)	
Marital status				$p>0.05$
Married	44	20 (45.4%)	24 (54.6%)	
Widowed-divorced-single	26	17 (65.3%)	9 (34.7%)	
Education				$p=0.003$
Illiterate	10	8 (80%)	2 (20%)	
Literate-Primary school	46	27 (58.6%)	19 (41.4%)	
Secondary-High school	14	2 (14.2%)	12 (85.8%)	
Chronic disease				$p>0.05$
Diabetes	12	6 (50%)	6 (50%)	
Hypertension	26	13 (50%)	13 (50%)	
Hypercholesterolemia	7	3 (42.8%)	4 (57.2%)	
Stroke	8	5 (62.5%)	3 (37.5%)	

*Pearson Chi-square.

All socio-demographic data of the individuals of the group of dementia were compared with the data of the group of healthy controls with the use of a Pearson Chi-square test at the $p<0.05$ level of statistical significance.

As shown in Table I, there is no statistically significant difference in the socio-demographic data between the dementia and control groups, with the exception of educational level. Chronic diseases, such as stroke, hypercholesterolemia, hypertension and diabetes, which may be confounding factors because they may affect the values of the laboratory parameters of the study, also did not differ between the dementia and control group.

The possible influence of chronic disease on the values of the parameters of the study was tested in each group separately with the use of a Mann Whitney *U*-test. The test indicated that in the control group, only stroke was positively related to the HCY value ($p=0.025$). In contrast, in the individuals with dimentia, there were no significant associations between diabetes, stroke, hypertension or hypercholesterolemia and the values of the parameters of the study.

HCY levels were, however, related with dementia. The mean (±SD) HCY value was 23.85±8.80 μmol/L in the dementia group, which is abnormal and significantly higher than the mean value in the control group 10.472±2.18 μmol/L ($p<0.01$).

Folate was significantly lower in the dementia group as compared to the group of normal controls at the $p<0.01$ level of statistical significance. The mean (±SD) values were 4.58±2.32 ng/ml and 11.024±2.48 ng/ml for the dementia and control groups, respectively.

Table II. *Biochemical factors in subjects with or without dimentia (n=70).*

Factor	Dementia group (mean±SD)	Controls (mean±SD)	*P*-value
Homocysteine (μmol/L)	23.85±8.80	10.472±2.18	$p<0.01$
Folate (ng/mL)	4.58±2.32	11.024±2.48	$p<0.01$
Vitamin B12 (pg/mL)	354.38±186.88	545.12±94.77	$p<0.01$

Dementia was related to the plasma level of vitamin B_{12}. The mean (±SD) value of vitamin B_{12} in the dementia group was 354.38±186.88 pg/mL, significantly lower than the mean value in healthy individuals (545.12±94.77 pg/ml; $p<0.01$). All the data from the laboratory analysis are presented in Table II.

Folate and vitamin B_{12} concentrations presented a positive linear correlation with the score at the MMSE rating scale (Spearman's rho= 0.343 and 0.412, respectively, $p<0.05$), indicating that folate and vitamin B_{12} levels were negatively correlated with the severity of cognitive decline (the lower the MMSE score, the larger is the burden of the disease). HCY presented a negative linear correlation with the score at the MMSE rating scale (Spearman's rho=–0.462, $p<0.05$), indicating that plasma HCY concentrations were positively correlated with the severity of cognitive decline.

Discussion

We attempted to investigate the possible associations between biochemical factors and dementia in elderly individuals. The diagnosis of dimentia is based almost solely on clinical examination and no laboratory tests are yet available in this respect, although considerable work is currently in progress with promising results. Numerous factors have been studied, reflecting the need and the value of biological markers for this disorder.

In this present study, the possible implications and associations of plasma levels of folate, vitamin B_{12} and homocysteine with dementia in a geriatric population were investigated. Of all sociodemographic data (age, gender, marital status, educational level), only the level of education differed between the individuals with dimentia and the controls. Educational level has been proposed to correlate inversely with the risk of dementia (28), though it should be noted that the majority of the population in this study was poorly educated.

Plasma HCY was significantly elevated in demented individuals compared to healthy controls. Increased homocysteine levels were significantly associated with adverse cognitive function in several previous population-

based studies (14, 15). Although, other studies have failed to replicate this association (29), it does appear possible that a positive relationship may exist. This relationship involves not only vascular dementia *via* the indisputable damages of vessels associated with HCY, but AD as well. A number of mechanisms has been proposed for the explanation of the relationship between elevated homocysteine level and dementia. Elevated HCY is an independent risk factor for cerebrovascular disease, which in turn, is a risk factor for cognitive decline and dementia (15). Moreover, HCY may either have direct neurotoxic effects (23) or promote β-amyloid toxic effects on neuronal cells (30).

In this study, the levels of vitamin B_{12} were significantly lower in the dementia group compared to those of the control group. This result is consistent with the results of other studies (12). Moreover, a causative role of vitamin B_{12} in dementia has been proposed (10), though other groups have failed to replicate this finding (16). Vitamin B_{12} deficiency may increase the risk of AD *via* elevated levels of HCY. B_{12} is necessary for the conversion of HCY to methionine and the accumulation of HCY may lead to neurotoxic effects. However, vitamin B_{12} deficiency is not associated with the pathological hallmarks of AD (10).

Folate was significantly lower in the dementia group. The association of lower plasma folate levels with cognitive impairment is supported by other studies (7, 9, 10), although negative results have also been reported (16, 19). Studies using animal models demonstrated that folate deficiency may promote neuronal degeneration (9) and sensitize neurons to amyloid β peptide toxicity, thereby, promoting AD (31). However, it is not clear whether the vulnerability and the subsequent damage are due to folate deficiency itself, which may induce DNA damage, or to the accumulation of HCY (9).

Future intervention in this field is important, as it is a cornerstone of human pathology. The identification of pathophysiological mechanisms for cognitive impairment in senior citizens may ease the management with fewer therapeutical interventions. Genetic studies, neuroimaging techniques and research for biological markers in general will help clarify the pathophysiology of dementia in late life and promote more effective therapeutic strategies.

Conclusion

The need for biological markers for early detection and prevention of the disease is indisputable. Unfortunately, there is still lack of such biological markers for dementia. The present work constitutes a pilot study in a limited population including a rather small number of participants. There was no intention of establishing biochemical markers ready for use in every-day practice and the biochemical parameters measured in this study suggest a possible association with cognitive impairment in the elderly. It cannot be estimated whether the measurement of plasma homocysteine, vitamin B_{12} or folate will be crucial in the future for the decision to initiate medication or not, but these factors may serve in the early identification of elderly individuals at risk for dementia and may also act as indicators of a modification in life-style need, in the context of detection and deceleration of dementia.

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