Original article

Serum folic acid is reduced in patients with Alzheimer's disease

Redução dos níveis séricos de ácido fólico em pacientes com a doença de Alzheimer

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Abstract

Background: Complex B vitamin deficiency has been associated to cognitive impairment and dementing disorders in the elderly. **Objective:** This work aims to assess whether patients with Alzheimer's disease (AD) and mild cognitive impairment (MCI) have lower levels of folic acid and cobalamin (vitamin B_{12}) compared to age and gender-matched controls. **Methods:** One hundred and forty six elderly subjects (40 AD, 56 MCI and 49 healthy older adults) were recruited for this study. Serum folic acid and vitamin B_{12} levels were measured by electrochemoluminescence. **Results:** Compared to MCI and healthy controls a statistically significant reduction in serum concentrations of folic acid in AD patients was found ($p = 0.02$). This result remained statistically significant after controlling for socio-demographic and cognitive performance variables ($p = 0.01$). No significant differences were found in serum concentrations of vitamin B_{12} in patients with AD, MCI and healthy controls. No significant changes in hematologic parameters were observed across these diagnostic groups. **Discussion:** The present study provides additional evidence that folic acid is reduced in patients with AD and reinforces the importance of nutritional changes, in particular the one-carbon metabolism, in the physiopathology of AD.

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Keywords: Folic acid, vitamin B_{12}, Alzheimer's disease, nutritional factors, one-carbon metabolism.

Resumo

Contexto: Deficiência de vitaminas do complexo B tem sido associada a deterioração cognitiva e quadros demenciais em idosos. **Objetivo:** Neste trabalho, foi avaliado se pacientes com doença de Alzheimer (DA) e com comprometimento cognitivo leve (CCL) apresentam níveis séricos de ácido fólico e cobalamina (vitamina B_{12}) menores que idosos controles. **Métodos:** Foram recrutados 146 idosos (40 com DA, 56 com CCL e 49 idosos controles) para este estudo. Os níveis séricos de ácido fólico e vitamina B_{12} foram avaliados pelo método de eletroquimioluminescência. **Resultados:** Os pacientes com DA apresentaram redução estatisticamente significativa nos níveis de ácido fólico em relação aos idosos com CCL e controles ($p = 0,02$). Esses resultados mantiveram-se estatisticamente significativos após controlar por variáveis sociodemográficas e desempenho cognitivo. Não se observaram diferenças estatisticamente significativas nos níveis de vitamina B_{12} nem em variáveis hematológicas entre os grupos. **Conclusão:** Esses resultados reforçam a importância de anormalidades em aspectos nutricionais, em particular do metabolismo de um-carbono, na fisiopatologia da DA.

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Palavras-chave: Ácido fólico, vitamina B_{12}, fatores nutricionais, metabolismo de um-carbono.

Introduction

In the recent years, there is a growing awareness of the relevance of homocysteine and related metabolic pathways in the pathophysiology, and, possibly, the prevention of Alzheimer's disease (AD)[1-3]. Homocysteine is a sulfur aminoacid derived from the metabolism of methionine by two major metabolic pathways: remethylation and transsulfuration[4]. These pathways are regulated by dietary ingestion of methionine, but also by other nutritional factors, in particular, folate and vitamin B_{12} levels. The latter mechanism stimulates the re-methylation pathway leading to the conversion of homocysteine to methionine[5]. High homocysteine levels are associated with increased oxidative stress, DNA methylation and apoptosis, being a risk factor to several diseases, including cardiovascular and neurodegenerative disorders[6-8].

Folic acid and vitamin B_{12} levels may serve as a surrogate marker of homocysteine levels in humans. Lower serum levels of both factors are significantly correlated with higher homocysteine levels in several studies[9-12]. Thus, dietary supplementation of folic acid and vitamin B_{12} would reduce homocysteine levels[13] and improve cognitive performance and prevent the development of AD in at risk elderly subjects[14]. Studies with animal models of AD suggested that folic acid and vitamin B_{12} supplementation reduced AD-related neuropathology and improved cognitive function in these models[15,16]. A positive association between high folate intake and reduced risk of AD has also been reported[17]. Despite these evidences, many observational studies and randomized clinical trials did not find a significant benefit of folic acid and vitamin B_{12} supplementation to reduced the progression of disease or improve cognitive performance[18-20].

Therefore, the aims of the present study are to assess differences of serum levels of folic acid and cobalamin (vitamin B_{12}) between patients with AD and mild cognitive impairment (MCI) as compared to age-matched healthy controls and to assess the relationship between these vitamins and cognitive performance in this test group.

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Methods

One hundred and forty six older adults were recruited to this study. They are part of a clinical cohort dedicated to the study of cognitive aging. Detailed description of the clinical and cognitive assessments and diagnostic procedures can be found elsewhere[21,22]. In brief, the patients underwent a comprehensive cognitive assessment which included the administration of the Rivermead Behavioral Memory Test (RBMT), the Fuld Object Memory Evaluation (FOME), the Trail Making Test A and B, semantic verbal fluency (category: fruit), the short cognitive test (SKT); the scores on the Mini-mental State Examination were used as a proxy measure of global cognitive performance.

The diagnosis of Alzheimer's disease (AD) was ascertained according to the NINCDS-ADRDA diagnostic criteria[23]. The diagnosis of mild cognitive impairment (MCI) was ascertained according to the Mayo Clinic criteria[24]. Elderly subjects with no evidence of cognitive impairment were regarded as healthy controls.

After clinical and cognitive assessments blood samples were taken from all subjects in the morning after 10 hours fasting. Serum folic acid and vitamin B_{12} levels were measured by electrochemoluminescence, as part of the routine laboratorial assessment.

Statistical analysis

Kolmogorov-Smirnoff tests were carried out to ascertained distribution normality of continuous variables. As the variables of interest in this study (i.e. folic acid and vitamin B_{12}) did not show a normal distribution ($p < 0.001$), we carried out non-parametric statistical tests (Kruskal-Wallis or Mann-Whitney tests, when applicable) to assess for differences between diagnostic groups. Qui-square tests were carried out to assess for differences in frequency distribution of dichotomous variables. Spearman correlation tests were done address the correlation between folic acid and vitamin B_{12} levels and clinical and cognitive variables.

Results

Tables 1 and 2 show the socio-demographic and cognitive performance variables for patients and controls. As expected, patients with AD and MCI were older, with less years of formal education and had worse cognitive performance. No significant differences in general nutritional status, as verified by total protein and hemoglobin levels, were observed among diagnostic groups (Table 3).

Patients with AD showed a significant reduction in serum concentrations of folic acid as compared to MCI subjects and healthy controls. No significant differences were observed between MCI and healthy controls. Also, no significant differences were observed in serum concentrations of vitamin B_{12} across the diagnostic groups (Table 3).

As age and education were significantly different between diagnostic groups, we carried out an analysis of covariance to control for the potential confounding effects of these variables on folic acid levels. After controlling for these factors, folic acid levels remained significantly reduced in patients with AD ($F = 4.5$, d.f. = 2, $p = 0.01$).

Spearman analysis showed a significant correlation between folic acid levels and memory scores (RBMT screening scores, rho = 0.190; $p = 0.03$), verbal fluency (rho = 0.247, $p = 0.006$) and Trail A (rho = -0.196; $p = 0.03$). There were no significant correlations with age, educational levels, hemoglobin or total protein serum levels, or scores on other cognitive tests.

Table 1. Socio-demographic data according to diagnostic group

	Diagnosis			p
	Controls (n = 49)	MCI (n = 56)	AD (n = 40)	
	Median [P25-P75]	Median [P25-P75]	Median [P25-P75]	
Gender (W / M)	40/9	45/11	28/12	0.36
Age	67 [64-70]	70 [67-75]	75 [71-77]	< 0.001
Years of education	15 [8-18]	8 [4-13]	4 [4-11]	< 0.001

W: woman; M: man. MCI: mild cognitive impairment; AD: Alzheimer's disease. P25: percentile 25; P75: percentile 75.

Table 2. Scores on cognitive tests according to diagnostic groups

	Diagnosis			p
	Controls (n = 49)	MCI (n = 56)	AD (n = 40)	
	Median [P25-P75]	Median [P25-P75]	Median [P25-P75]	
MMSE	29 [27-30]	27 [26-29]	18 [14-22]	< 0.001
RBMT (screening score)	11 [9-12]	8 [6-9]	2 [0-4]	< 0.001
RBMT (profile score)	22 [21-24]	18 [16-20]	6 [1-11]	< 0.001
FOME (total score)	45 [44-47]	40 [37-44]	18 [12-31]	< 0.001
FOME (delayed recall)	10 [9-10]	9 [8-10]	3 [2-6]	< 0.001
Verbal fluency (fruits)	15 [13-17]	12 [10-14]	8 [7-11]	< 0.001
Trail A (s)	49 [40-55]	65 [54-86]	101 [66-160]	< 0.001
Trail B (s)	97 [78-133]	188 [141-219]	240 [207-268]	< 0.001
SKT	2 [1-3]	3 [2-5]	11 [8-16]	< 0.001

MMSE: Mini-Mental State Examination; RBMT: Rivermead Behavioral Memory Test; FOME: Fuld Object Memory Evaluation; SKT: Short Cognitive Test. P25: percentile 25; P75: percentile 75.

Table 3. Folic acid, vitamin B_{12}, hemoglobin and total protein levels according to diagnostic groups

	Diagnosis			p
	Controls (n = 49)	MCI (n = 56)	AD (n = 40)	
	Median [P25-P75]	Median [P25-P75]	Median [P25-P75]	
Folic acid (ng/ml)	9,20 [5,60-13,90]	9,40 [6,50-14,15]	6,20 [4,05-11,80]	0,02
Vitamin B_{12} (pg/ml)	368,00 [251,00-488,00]	402,50 [300,50-735,50]	354,50 [214,00-451,00]	0,09
Hemoglobin (g/dl)	14,00 [13,10-14,60]	14,00 [13,35-14,60]	13,80 [13,05-15,00]	0,9
Total protein (g/dl)	7,2 [7,1-7,5]	7,2 [7,0-7,4]	7,4 [7,5-7,7]	0,4

P25: percentile 25; P75: percentile 75.

Discussion

In our study, we found a significantly lower folic acid levels in patients with AD as compared to subjects with MCI and healthy older adults, which was independent of age and educational level. No differences were observed between elderly controls and MCI subjects. In addition, lower folic acid level was correlated with worse cognitive performance, in particular in memory, language and psychomotor speed. It is important to note that, despite lower folic acid levels in AD patients, the median values was above the lower range of laboratorial reference values (i.e. 4.2 to 19.9 ng/ml). Thus, these patients did not show a clinically significant vitamin deficiency that could explain the present results. These results could not be better explained by differences in nutritional status. Thus, our results suggest that reduction in folic acid levels may be a metabolic feature in the pathophysiology of AD, with a possible negative effect on cognitive performance. Because patients with MCI did not display a similar reduction in the concentrations of folic acid, we speculate that this abnormality may be only observed in later stages of the neurodegenerative process (i.e. when subjects already present with clinically manifest dementia). Nevertheless, we must take into account that the lack of difference between patients with MCI and normal controls may be a consequence of the biological heterogeneity of the MCI group, which was recruited according to clinically-oriented diagnostic criteria.

Our results are in accordance with previous reports in the literature[25,26]. These studies found that the lower folic acid levels, higher the risk of AD in community-based studies. In contrast to our results, these studies also found a significant association between lower vitamin B_{12} levels and AD. Methodological differences, such as sample size and study setting, may in part explain these differences.

The actual mechanism that leads to a reduction of folic acid in AD remains to be established. One possible explanation is the evidence that there is an inverse relationship between folic acid and homocysteine levels (Morris, 2003). Folate is a co-factor in one-carbon metabolism during which it promotes the regeneration of methionine from homocysteine, a highly reactive sulfur-containing amino acid. Thus, patients with low folic acid levels may present with increased homocysteine levels what in turn is neurotoxic and lead to neurodegenerative changes[27]. This is reinforced by the evidence that high homocysteine levels are independently associated to AD[28,29]. So low folate status and elevated homocysteine besides the well-established roles on induction of DNA damage, increase the generation of reactive oxygen species and contribute to excitotoxicity, mitochondrial dysfunction and apoptosis. Also, low folate levels determine an imbalance between glycogen synthase kinase 3-beta (GSK-3β) and protein phosphatase 2A (PP2A) activity, leading to abnormal hyperphosphorylation of tau in AD[30]. Finally, methionine may be converted to S-adenosylmethionine (SAM), the principal methyl donor in most biosynthetic methylation reactions. Low folate leads to lower levels of SAM what in turn may increase DNA methylation and leading to changes in the epigenetic control of learning and memory acquisition[31]. Therefore, low folate level, as observed in our study, have several negative effects to neuronal functioning that are related to some extend to the pathophysiology of AD[32].

In conclusion, our study showed a significant reduction in folate levels in patients with AD as compared to healthy controls. Our results highlight the importance of the assessment of nutritional aspects in AD and also for a possible role of folate deficiency in its physiopathological features. Despite previous clinical trial did not find a significant effect of folate supplementation to improve cognition on patients with AD, we can rule out potential beneficial effects of long-term folate supplementation for reducing the risk of AD.

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Conflict of interest

None to declare.

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The funding sources did not have any role in the study conception, design, data analysis and in the elaboration of the manuscript.

Authors' contributions

Cesar C. Almeida designed the study, collected data, carried out the statistical analysis and wrote the manuscript. Helena P. Brentani and Orestes V. Forlenza analysed the data and wrote the manuscript.

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