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Homocysteine concentrations in the cognitive progression of Alzheimer's disease

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ABSTRACT

Objectives: Hyperhomocysteinemia in Alzheimer's disease (AD) is widely reported and appears to worsen as the disease progresses. While active dietary intervention with vitamins B12 and folate decreases homocysteine blood levels, with promising clinical outcomes in Mild Cognitive Impairment (MCI), this so far has not been replicated in established AD populations. The aim of the study is to explore the relationship between hyperhomocystenemia and relevant vitamins as the disease progresses.

Methods: In this longitudinal cohort study, 38 participants with mild to moderate AD were followed for an average period of 13 months. Plasma folate, vitamin B12 and homocysteine concentrations were measured at baseline and at follow-up. Dietary intake of B vitamins was also measured. Spearman's correlations were conducted by homocysteine and B vitamin status.

Results: As expected, cognitive status significantly declined over the follow-up period and this was paralleled by a significant increase in homocysteine concentrations ($p = 0.006$). However, during this follow-up period there was no significant decline in neither dietary intake, nor the corresponding blood concentrations of vitamin B12/folate, with both remaining within normal values. Changes in blood concentrations of B vitamins were not associated with changes in homocysteine levels ($p > 0.05$).

Conclusion: In this study, the increase in homocysteine observed in AD patients as the disease progresses cannot be solely explained by dietary and blood levels of folate and vitamin B12. Other dietary and non-dietary factors may contribute to hyperhomocysteinemia and its toxic effect in AD, which needs to be explored to optimise timely intervention strategies.

1. Introduction

Dementia is a common disorder with its prevalence set to rise worldwide. In the absence of a cure there is a growing need to identify treatments that can slow the cognitive progression of the disease, thus enabling those with dementia to retain appropriate functional abilities for longer. Currently, there are only a small number of symptomatic medications licensed for the treatment of Alzheimer's disease (AD), the most prevalent type of dementia. Importantly, the effectiveness of these licensed medications are often considered limited (Casey et al., 2010).

Abnormal elevation of homocysteine levels has been implicated as a marker for AD. Hyperhomocysteinemia is associated with increased cognitive decline in healthy older adults with higher risk of cognitive impairment (e.g. Morris, 2012; Smith et al., 2010; Vogel et al., 2009). Apart from a few notable exceptions, most studies report mild to moderate hyperhomocysteinemia in dementia populations compared to healthy controls (for a review see Zhuo et al., 2011). However, research into the specific role of homocysteine on cognitive decline in a population with established AD is limited. Although not consistently reported (e.g. Huang et al., 2010), studies have found that homocysteine levels in AD can predict rates of cognitive decline (Huang et al., 2013; Oulhaj et al., 2010).

It has therefore been of interest to establish whether reducing homocysteine levels can have a beneficial effect on the slowing down of AD progression. A key avenue of research is the supplementation with vitamin B12 and folate. These vitamins act as cofactors for the methylation of homocysteine to methionine, and therefore in the absence of these dietary nutrients homocysteine concentrations increase. It has

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been estimated that 65% of the hyperhomocysteinemia cases among the Framingham elderly population-based cohort could be accounted for by inadequate folate, or, to a lesser extent, inadequate vitamin B12 or vitamin B6 status and intake (Selhub et al., 1993). It has even been shown that B vitamins taken at the earliest stages of the disease (i.e. Mild Cognitive Impairment (MCI)) are able to slow down the rate of brain atrophy (Smith et al., 2010) as well as the cognitive decline (de Jager et al., 2012), and may reduce conversion rates into dementia (Blasko et al., 2013). Despite this, there is little evidence that increasing the intake of these vitamins are able to combat cognitive decline later in the disease course, i.e. among participants with established AD. Serum levels of vitamin B12 have been found to have no association with cognitive progression in AD populations (Huang et al., 2010; Oulhaj et al., 2010; Small et al., 1997a, 1997b; Tu et al., 2010). Randomized controlled trials (RCTs) in AD populations have found that relevant vitamin treatments are unable to attenuate cognitive progression in the samples as a whole (Aisen et al., 2008; Kwok et al., 2011; Sun et al., 2007). Interrogation of these findings suggest that B vitamin supplementation may still have a role in attenuating cognitive decline in AD only in the presence of particularly elevated homocysteine levels. For example, Kwok and colleagues found that participants who received B vitamin treatment with elevated levels of homocysteine (13 μmol/L or greater) had significantly smaller decline in the construction domain of Mattis Dementia Rating Scale (MDRS) than those receiving placebo (Kwok et al., 2011). Interestingly, participants in another study that had more modest levels of homocysteine (mean = 9.2 μmol/L, SD = 3.2) at the onset of the trial did not report any positive effects on cognition, while a subgroup with mild AD showed some benefit of the treatment (Aisen et al., 2008).

Discerning the exact role of homocysteine and B vitamins on cognitive decline is complicated, as hyperhomocysteinemia is only one of many contributing markers in the disease progression. The neurotoxicity of homocysteine and its direct effect on brain atrophy has been established (Madsen et al., 2015). Unlike the early indications from MCI, lowering homocysteine levels in established AD may not produce the same clinical benefits as research so far has shown. Other non-dietary factors may also contribute to hyperhomocysteinemia and moderate its toxicity. Longitudinal studies assessing the relationship between homocysteine concentrations and cognitive decline in established AD, and its possible association with both dietary and blood vitamin B12 and folate levels, are limited (Clarke et al., 1998; Oulhaj et al., 2010).

The aim of this study was to establish whether the extensively reported hyperhomocysteinemia in AD as the disease progresses is associated with dietary and blood levels of B vitamins. In line with current knowledge, we hypothesised that blood homocysteine levels would significantly increase over the follow-up period. In addition, we hypothesised that this increase in homocysteine is explained by an associated decrease in vitamin B levels.

2. Materials and methods

2.1. Participants

All participants were recruited in Sussex (UK) memory assessment clinics and had mild to moderate AD (MMSE > 12). The mean age of AD participants was 81.3 years (SD = 6.0). The inclusion and exclusion criteria for this study have previously been reported elsewhere (Farina et al., 2016). In brief, eligible participants had previously been clinically assessed using the International Statistical Classification of Diseases, 10th revision (ICD-10; World Health Organization, 1992), and received a diagnosis of probable dementia of Alzheimer's type. AD patients all had a personal consultee (relative or friend), and were either clinically or self-referred. For inclusion in the study described here, participants were also required to provide a blood sample on two occasions so that homocysteine concentrations could be measured. Ethical approval was obtained from a National Research Ethics Service Committee.

2.2. Neuropsychological testing and dietary data

Demographic data including age, gender and ethnicity were recorded. As previously reported (see Farina et al., 2016), a battery of neuropsychological tests and the Food Frequency Questionnaire (FFQ) were completed by participants and lasted approximately 2–3 h. The Addenbrooke's Cognitive Examination Revised (ACE-R; Mioshi et al., 2006) was used to provide a standardised measure of dementia severity. The ACE-R total score was also used as a comprehensive measure of global cognitive status. The Cornell Scale of Depression in Dementia (CSDD; Alexopoulos et al., 1988) was used to screen for the potential presence of major depression. IQ was estimated using the National Adult Reading Test (NART; Nelson, 1982).

Dietary intake of nutrients were estimated using the EPIC FFQ (Bingham et al., 2007). The questionnaire was completed by the carer of the participant, who described the average dietary intake of the participant over the past year. Once collected, data was analysed by the Department of Nutritional Sciences, University of Surrey, UK. Nutritional values for individual foods were estimated and summed. Nutrient intakes were expressed as percentages of the age and gender appropriate Recommended Nutritional Intakes (RNIs).

2.3. Biochemical assays

Plasma homocysteine, vitamin B12, and folate were analysed at the Department of Pharmacology, University of Oxford. Plasma concentrations of total Cobalamin (B12) and folate were measured by automated (Perkin-Elmer MultiProbe 11 liquid handling system, Perkin-Elmer Life and Analytical Sciences) microbiological assays using *Lactobacillus leichmanii* and L. *casei*, respectively (Kelleher and Broin, 1991; Molloy and Scott, 1997). Between-day coefficients of variation for B12 were 7.1%, and 7.4% for folate. Total plasma homocysteine was analysed by liquid-chromatography according to a modified protocol described previously (Refsum et al., 2004), using a QTRAP 5500 (AB Sciex, Framingham, Massachusetts, US) coupled to a Prominence LC-$20AD_{XR}$ binary pump (Shimadzu, Kyoto, Japan). In short, 10 μL of plasma was added to an equal volume of an internal standard mix containing isotopically labelled homocysteine. Samples were neutralised using an ammonia solution before reduction with dithioerythriol at room temperature for 15 min. Plasma proteins were precipitated with perchloric acid (4% v/v) and cleared by centrifugation. Supernatant was diluted 1:10 in water/sodium 1-heptane sulfonate [1 M]/perchloric acid [20%, 3.3 M] 5/3/1 v/v/v, and injected onto a Kinetex C18 column (30 × 4.6 mm, 2.6 μm, Phenomenex, Torrance, CA, US) at a flow rate of 0.8 mL/min. Gradient elution was employed starting at 100% mobile phase A (water + 0.05% formic acid), with a final composition of 40% A and 60% mobile phase B (methanol + 0.05% formic acid). Data acquisition and analysis were performed with Analyst 1.6.1 (AB Sciex, Framingham, Massachusetts, US). Quantitation was based on the ratio of analyte peak area/internal standard peak area against a linear calibration curve with a 1/x weighting. The coefficient of variation for was 4.0%.

2.4. Statistical analysis

Descriptive statistics (e.g. means and frequencies) were reported for the participant demographics. All findings reported were of participants that were able to provide a sample of blood at baseline and at follow-up so that plasma homocysteine concentrations could be analysed. A series of Spearman's Rho correlations were conducted between baseline measures of B vitamin status and plasma homocysteine levels to determine the relationship between them. Additional Spearman's Rho correlations were run between B vitamin status and homocysteine