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Research report

Age dependent levels of plasma homocysteine and cognitive performance

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HIGHLIGHTS

- Correlation between homocysteine and cognitive function largely unknown in India.
- Homocysteine levels negatively correlated with cognitive performance.
- Homocysteine levels negatively correlated with folate and vitamin B12 levels.
- B vitamin supplements in this Indian population may improve cognitive function.

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ABSTRACT

Elevated plasma homocysteine (hcy) levels, also known as hyperhomocysteinemia (hhcy), have been associated with cognitive impairment and neurodegenerative disorders. Hhcy has been attributed to deficiency of B vitamins which can adversely affect the brain and result in memory loss and poor attention power. Monitoring hcy levels and the use of vitamin supplementation to treat hhcy may therefore prove advantageous for the prevention and management of cognitive impairment. With this in consideration, we measured plasma hcy, folate and vitamin B12 levels in 639 subjects from different age groups in two sub-regions of India. Cognitive function was also measured using attention span and immediate and delayed memory recall tests. Depression scores were obtained using the Beck Depression Inventory-II and functional impairment was assessed using the functional activities questionnaire (FAQ) score. As hhcy has also been linked to inflammation, plasma levels of high sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6) were also measured. The results demonstrated significant negative correlations between hcy levels and folic acid levels, vitamin B12 levels and cognitive performance (attention span and delayed but not immediate memory recall) along with significant positive correlations between hcy levels and depression scores and hsCRP (but not IL-6) levels. A positive correlation was also observed between hcy levels and FAQ scores, however this was not found to be significant. Based on these results, folic acid and vitamin B12 intervention in people with elevated hcy levels in India could prove to be effective in lowering hcy levels and help maintain or improve cognitive function.

1. Introduction

Elevated plasma total homocysteine (hcy), also referred to as hyperhomocysteinemia (hhcy), is associated with cognitive impairment [1] as well as with an increased risk of Alzheimer's disease (AD), vascular dementia, and cerebrovascular stroke [2–5]. Vitamin B6 and B12 and folate play an important role in hcy metabolism (remethylation or trans-sulfuration) [6], it is therefore unsurprising that nutritional status can play a pivotal role in the development of hhcy. In this context vegetarian diets have been found to be associated with higher plasma hcy levels compared to non-vegetarian diets [7–9]. Hcy levels also increase with age and are higher in males than females [10].

There are several mechanisms that may account for the increased risk of neurological and cardio-vascular disorders in

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people with hhcy. Hcy can act as a glutamate receptor agonist and thereby hhcy may contribute towards excitotoxicity in the brain and induce oxidative stress [11]. Hcy can also induce inflammation by enhancing oxidative stress or through nuclear factor kappa-β activation [12]. Several studies have also associated hhcy as well as low folate and vitamin B12 levels with a decrease in DNA methylation which in turn can alter gene expression and cause DNA damage. This decrease in DNA methylation has been linked to reduced S-adenosyl methionine (SAM) levels due to low folate and vitamin B12 levels and increased S-adenosyl homocysteine (SAH) levels due to high hcy levels [11–13].

Several studies have demonstrated the effectiveness of B vitamin supplementation in lowering elevated plasma hcy levels; however there have been mixed reports regarding the clinical benefits of such therapy on cognitive function [2,4,5]. Stott et al., found no benefits of folic acid plus vitamin B12 treatment for 12 weeks on cognitive function in elderly subjects with vascular diseases [14]. A longer 4 months treatment period with B vitamins (cyanocobalamin plus folic acid plus vitamin B6) also failed to improve cognitive performance in the elderly [15]. In another study, no significant cognitive improvements were observed following B vitamin treatment (vitamin B12 plus folic acid or vitamin B12 alone) for 24 weeks in older subjects with mild vitamin B12 deficiency [16]. Longer term (2 years) treatment with B vitamins (folic acid plus vitamin B6 and B12) also failed to improve cognitive performance in healthy older subjects with high hcy levels (>13 μmol/L) [17]. Similarly, B vitamin treatment for 2 years did not improve cognitive function in hypertensive older men or reduce the risk of cognitive impairment and dementia [18].

On the other hand, some studies have reported on the beneficial effects of hcy lowering B vitamin therapy on cognitive performance. Positive effects of short term treatment with folic acid, vitamin B6 or vitamin B12 on memory performance have been reported in young, middle aged and older women [19]. Folic acid intervention for 60 days in elderly with both low plasma folate levels and memory complaints also improved memory deficits [20]. Longer term (>6 years) B vitamin treatment (folic acid plus vitamin B6 and B12) resulted in some benefits on cognitive performance in older women who had a low dietary intake of B vitamins and were at high risk for cardiovascular disease [21]. Another study reported a positive effect of folic acid treatment for 3 years on age related cognitive decline in older subjects with high hcy but normal vitamin B12 levels [22].

Several studies have reported elevated hcy levels in AD patients [4]. However these have been accompanied by reports of the lack of benefits of hcy lowering therapy in AD patients, including folic acid plus vitamin B12 treatment for 12 weeks [23] or 24 months [24] and folic acid plus vitamins B6 and B12 treatment for 18 months [25] or 26 weeks [26]. This however is unsurprising as the dementia stage of AD is associated with well-advanced pathologies and targeting AD in the earlier stages, prior to the onset of dementia, presents a more realistic opportunity to effectively treat the disease [27]. In support of this, treatment with B vitamins effectively lowered hcy levels, the rate of brain atrophy, and the rate of cognitive decline in elderly with both mild cognitive impairment and high baseline hcy levels [28–30]. Nilsson et al. also reported cognitive improvements in mild to moderate (but not severe) dementia patients with hhcy following B vitamin treatment [31].

Differences between various aspects of the studies investigating the cognitive benefits of hcy lowering therapy, including the ethnicity, nationality, age, disease status and baseline plasma hcy, folate, vitamin B6 and vitamin B12 levels of the subjects have made it difficult to generalize their findings. Differences in the tools used to measure cognitive performance, the nature and duration of the hcy lowering treatment, and on occasions inadequate sample sizes used in these studies have all added to the complexity of generalizing their findings. Nevertheless there have been encouraging signs that subsets of patients with hhcy can benefit from hcy lowering therapy (e.g. low folate levels coupled with hhcy) [32]. The timing of intervention and duration of therapy is also likely to be important, as supported by a study in Sweden which reported that higher hcy levels in midlife were associated with an increased risk of AD later in life [33].

The current study was designed to investigate the relationship between plasma hcy levels and cognitive function, plasma vitamin B12 levels, and plasma folate levels across different age groups in India. The results will help determine whether hcy lowering therapy may be useful to maintain healthy cognitive function and prevent neurodegeneration in this population.

2. Materials & Methods

2.1. Subjects

Recruitment of subjects between the ages of 18–78 years was carried out as part of a field study near Kattankulathur and Kanchipuram in Tamil Nadu (South India) and near Varanasi (Khojwa, Sundarpur, and Jaunpur) in Uttar Pradesh (North India). Exclusion criteria included – high glycated haemoglobin (>10%), high postprandial glucose (>300 mg/dl), chronic renal failure, congestive cardiac failure, and evidence of malignancy or tuberculosis. Demographic information was collected for the following parameters: age, gender, and body mass index (BMI). Further biochemical and cognitive measures were determined as described below. The study was approved by ethical committees of both the organizations (Banaras Hindu University, Varanasi, India and SRM University, Chennai, India; Ref. no. dean/2009-10/1412 dt11.2.2010).

2.2. Neuropsychological tests

Memory performance was determined using immediate and delayed word recall scores using an electronic Memory Span apparatus (Medicaid systems, Chandigarh, India). Attention span was measured using an electronic Attention Span apparatus (Medicaid systems, Chandigarh, India). Measurement of functional impairment was performed using the Functional Activities Questionnaire (FAQ) [34]. Depression scores were determined using the Beck Depression Inventory-II [35].

2.3. Biochemical tests

Plasma levels of interleukin-6 (IL-6), high sensitivity C-reactive protein (hsCRP), folic acid, vitamin B12, and total hcy were determined using commercially available ELISA kits (ENZO Life science).

2.4. Statistical analyses

All the values were calculated as mean ± SD (standard deviation). Correlation of total hcy with other biological and cognitive parameters was calculated by Spearman's correlation. Mean values obtained for the different subgroups were compared using one-way ANOVA. Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 11.5 software.

3. Results

A total of 639 participants took part in the study, consisting of 415 (64.9%) male and 224 (35.1%) female subjects between the ages of 25–75 years. Males had significantly higher hcy levels (12.73 ± 4.14 μmol/L versus 10.98 ± 3.82 μmol/L; $P < 0.01$) as well