Homocysteine, vitamin B 12 and folate in Alzheimer’s and vascular dementias: The paradoxical effect of the superimposed type II diabetes mellitus condition

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Abstract

Background: Increased concentration of plasmatic homocysteine (tHcy) and decreased vitamin B 12 (B12) and folate (FOL) are associated with Alzheimer’s (AD) and vascular (VaD) dementias, with type II diabetes mellitus (DM), and reported as risk factors of these diseases.

Abbreviations: tHcy, total homocysteine; B12, vitamin B 12; FOL, folate; AD, dementia of the Alzheimer type; VaD, vascular dementia; DM, type II diabetes mellitus; AD+DM, dementia of the Alzheimer type plus type II diabetes mellitus; VaD+DM, vascular dementia plus type II diabetes mellitus; CTR, controls; ANOVA, analysis of variance.

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**Methods:** The sample \((n=122;\ \text{males}=60;\ \text{mean age}=73 \pm 7 \text{ years})\) comprised AD and VaD patients without DM, with a concomitant DM (AD+DM, VaD+DM), DM alone and controls (CTR), resulting in 6 groups. tHcy, B12 and FOL were determined in duplicate.

**Results:** The one-way ANOVA yielded significant differences between groups for all variables: tHcy \(p<10^{-12};\ B12\ p<10^{-3};\ \text{FOL} \ p<10^{-4}\). Significance for comparisons between groups was set at \(\alpha=0.05\), using the Bonferroni’s statistic. The comparisons: DM vs. CTR, AD+DM vs. AD, VaD+DM vs. VaD, and DM demented vs. DM non-demented resulted significant for all variables, except for B12 in 2 comparisons.

**Conclusions:** In demented and control subjects, tHcy and FOL exhibit extreme differences, not so marked between DM and controls. Demented patients with concomitant diabetes are closer to controls than their non-diabetic counterparts. Diabetes affects tHcy and FOL values, which are changed with opposite sign to non-demented. These results suggests a paradoxical phenomenon when diabetes is superimposed to dementias.

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**1. Introduction**

Increased blood concentrations of plasma homocysteine (tHcy), often associated with low normal folate (FOL) and vitamin B 12 (B12) concentration are common in vascular dementia (VaD) and dementia of the Alzheimer’s type (AD). A moderate increase in plasma tHcy constitutes an independent risk factor of VaD, also associated with metabolic disorders in diabetes and with degenerative cognitive deterioration. High concentration of tHcy have been identified as risk factors for vascular disease and potentially for dementia and depression [1–4], in addition to cardiovascular disease. In association with the non-insulin-dependent type II diabetes (DM) it is a risk factor for vascular disease [5] and might also worsen the neurological course of ischemic cerebral infarctions and silent brain lesions.

The mechanisms behind these relationships are not clear, but enough evidence exists that the increased concentration of tHcy interferes with different coagulation factors and endothelial mechanisms, the individuals becoming prone to vascular lesions.

Methionine from the diet and from the catabolism of proteins in the cells is the only source of homocysteine through 2 metabolic stages: transulfuration and remethylation. The most common cause of increased tHcy is a deficiency of FOL or B12, but low concentration of FOL or B12 do not explain the increased tHcy concentration in Parkinson’s disease patients [6], where it was found to be associated with the use of L-dopa [7]. In diabetic patients, hyperinsulinemia has been found to increase tHcy [8]; in the pathogenesis of hyperhomocysteinemia in diabetes, the mutation in the C677T methylene-tetrahydrofolate reductase could contribute to increased concentration of tHcy [9].

The low plasma concentration of vitamins B6, B12 and FOL are normalized when supplemented with vitamins, suggesting that the pathologies related with hyperhomocysteinemia might be prevented with a vitamin-rich diet, a fact yet to be proved in extended trials.

Since many hereditary, mutational, nutritional and pharmacological factors might induce an increase in plasmatic tHcy, the presence of hyperhomocysteinemia is not indicative of a particular condition.

In this study, the profile studied comprises measurements of tHcy, B12, and FOL in plasma. We studied demented patients using diabetes as a second criterion, testing the following hypotheses: (a) increased concentration of tHcy and decreased concentration of B12 and FOL are linked with diabetes; (b) when associated with AD and VaD diseases the variations due to the diabetes condition are added to the pathological concentration observed in AD and VaD patients, rendering them more abnormal; (c) the profiles of demented plus diabetes patients could be distinguished.

**2. Materials and methods**

**2.1. Patients and controls**

The initial population of 130 subjects consisted of healthy controls (CTR), DM patients, and de-
mented patients of the AD and VaD types with and without associated DM. CTR subjects were selected by age and sex to reflect the general gender and age distribution of the diseased groups. Out-patients and controls were from Caucasian origin, recruited from the Neurology Service and the Diabetes Unit of the Hospital Sirio-Libanés, the Hospital General de Agudos Juan A. Fernández and the FACENE.

Patients and controls were included in the study accordingly with accepted neurological criteria for each group: the NINCDS-ADRDA criteria [10] for AD patients, the NINDS-AIREN criteria [11] for VaD patients, the ADA and the WHO criteria’s [12] for DM patients, and the DSM IV criteria [13] for the non-demented control subjects. All subjects underwent neurological, psychiatric, physical examination and comprehensive sets of neurological tests, and recruited provided that they had not a history of smoking and treatments or supplementation with vitamins in the last 5 years. VaD patients were studied with the Mattis scale [14], AD patients were studied with the ADAS scale [15], functional assessment and depression for all patients was conducted using CDR [16] and Hamilton tests [17]. Since being recruited and until data analysis (a period of 17 months for the first recruited subject), all subjects were controlled every other month.

Eight subjects were excluded from the initial population; further details are given in a previous publication [18]. The 122 individuals retained (60 males, mean age 73 years, S.D. 7 years) resulted in 6 groups: 19 CTR subjects, 18 DM patients, 29 non-diabetic AD patients, 19 diabetic AD patients (AD + DM), 19 non-diabetic VaD patients, and 18 diabetic VaD patients (VaD + DM).

Mean ± S.D. for the onset time were: for AD patients 6.5 ± 1.2 (range: 4–9) years; for VaD patients 5.6 ± 1.9 (range: 2–7) years; for DM patients 10.4 ± 3.8 (range: 4–21) years. All patients with dementia syndromes present CDR stages into the range between 1 and 2: for AD, 48% and 52%; for AD + DM, 43% and 57%; for VaD, 40% and 60%; for VaD + DM, 37% and 63%, respectively.

Within the DM population, 40 patients were on sulfonylurea medication (72.73%): 27 (48.10%) on glibenclamide, 8 (14.54%) on glimepiride, and 5 (9.09%) on glicazide; 7 were on metformin medication (12.73%); 4 were on α-glucosidase inhibitor medication (7.27%); and 4 received no medication but only diet (7.27%).

2.2. Blood sampling

Small volumes of venous blood were obtained with written informed consent from healthy volunteers and patients, after a fasting period of 12–14 h. Each subject contributed one sample, which was processed in duplicate and the mean used. The analysis of the differences of the duplicates indicated that this source of variability is non-significant. Venous blood samples were processed within 45 min of extracted at room temperature, including coagulation and centrifugation. If measurements had to be delayed, plasma was kept at 4–6 °C.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>tHcy&lt;sup&gt;a&lt;/sup&gt;</th>
<th>B 12&lt;sup&gt;b&lt;/sup&gt;</th>
<th>FOL&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Age&lt;sup&gt;d&lt;/sup&gt;</th>
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<td><strong>CTR, n = 19 (12M/7F)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
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<td>966.32</td>
<td>13.05</td>
<td>73.89</td>
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<td>1.88</td>
<td>346.79</td>
<td>3.96</td>
<td>8.87</td>
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<td>671.75</td>
<td>10.55</td>
<td>66.94</td>
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<td>306.77</td>
<td>4.11</td>
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<td><strong>AD, n = 29 (6M/23F)</strong></td>
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<td>18.35</td>
<td>633.86</td>
<td>7.89</td>
<td>73.35</td>
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<td>295.91</td>
<td>3.17</td>
<td>5.36</td>
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<tr>
<td>Mean</td>
<td>16.79</td>
<td>561.58</td>
<td>8.65</td>
<td>73.21</td>
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<td>277.64</td>
<td>2.94</td>
<td>6.19</td>
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<tr>
<td>Mean</td>
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<td>555.16</td>
<td>7.25</td>
<td>74.32</td>
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<tr>
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<td>3.35</td>
<td>5.14</td>
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<td>586.39</td>
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<td>72.56</td>
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<td>S.D.</td>
<td>3.81</td>
<td>224.61</td>
<td>4.20</td>
<td>5.90</td>
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</table>

<sup>a</sup> tHcy is expressed in μmol/l.
<sup>b</sup> B 12 is expressed in pg/ml plasma.
<sup>c</sup> FOL is expressed in ng/ml plasma.
<sup>d</sup> Age is expressed in year.
2.3. Homocysteine (tHcy) assay

The tHcy was determined using the IMx Homocysteine technique, from ABBOTT Diagnostics Division; it is a fluorescence polarization immunoassay (FPIA) based on the highly selective enzymatic conversion of homocysteine to S-adenosyl-L-homocysteine, which is then recognized by a monoclonal antibody [19]; requires a sample volume of 50 μl and the concentration of tHcy is expressed in μmol/l.

2.4. Vitamin B12 (B12) assay

The B12 was determined by the AxSYM B12 technique, from ABBOTT Diagnostic Division, an enzyme immunoassay based on microparticles cov-
dered with intrinsic factor (MEIA), for the quantitative determination of vitamin B12 in human serum or plasma using the AxSYM system [20]. The results were expressed in pg B12/ml plasma.

2.5. Folate (FOL) assay

The FOL was determined by the AxSYM Folate technique, from Abbott, which is a ionic capture assay used for quantitative determination of folate in serum, plasma or human erythrocytes by means of the AxSYM analyzer [21]. The results are expressed in ng FOL/ml plasma.

2.6. Statistical analysis

A one-way Analysis of Variance (ANOVA) [22] was performed on each variable and the Bonferroni statistic was employed to compare the mean values of groups of patients and controls. The method allows testing several differences of interest, ensuring that the overall significance level is maintained at \( \alpha = 0.05 \) raising the critical value used in the individual tests. In this case the critical value is \( t_c = 2.5233 \), used to test the significance of the planned comparisons.

For display and comparison, all data were standardized subtracting from each individual measurement the overall mean, calculated across all treatments, and dividing by the overall S.D. (i.e., all transformed variables have mean = 0 and S.D. = 1). After this change of scale the differences between groups are expressed, without units, in terms of S.D.

3. Results

The basic statistics of the 6 experimental groups are presented in Table 1, together with the mean and S.D. obtained for tHcy, B12 and FOL. Table 2 shows the ANOVA results for each variable, and the Bonferroni statistics calculated for the planned comparisons: DM vs. CTR, AD + DM vs. AD, VaD + DM vs. VaD, and diabetes effect on non-demented vs. diabetes effect on demented subjects. The ratios of males/females in each comparison were almost similar (0.55 vs. 0.63, 0.36 vs. 0.21, 0.72 vs. 0.58, and 0.55 vs. 0.54, respectively). Differences between groups resulted significant with low probabilities for all variables.

While the overall error in the ANOVA is based on 116 degrees of freedom, the number of patients in each group is relatively short (around 20). Anyhow, the very low probabilities associated with the differences between groups and the robustness of the ANOVA technique ensure that the results are not an artifact due to small sample size.

![Fig. 2. Profiles of the differences between diabetic and non-diabetic groups. DM group–CTR group: –●--; (AD + DM) group–AD group: –■--; (VaD + DM) group–VaD group: –▲--. Points are joined for visualization, not meaning a functional relationship between axes.](image)
Concentration of tHcy decreased progressively and significantly among groups as:

VaD > AD + DM > VaD + DM > DM > CTR.

With regards to B12 and FOL the order is practically reversed, respectively:

CTR > DM > AD + DM > VaD + DM > AD + DM > VaD.

**Fig. 1** shows the standardized profiles of each group, and **Fig. 2** the effect of the superimposed diabetes as the differences between diabetic groups and non-diabetic counterparts.

### 4. Discussion

It has been suggested that an increase in tHcy concentration precedes the development of dementia [23]. The mechanism underlining this interpretation is unknown; meanwhile the hyperhomocysteinemia has been related with brain microangiopathy, endothelial dysfunction, impairment of the nitric oxide activity, increase in oxidative stress and neuronal apoptosis, all mechanisms implied in brain aging.

It has been found that those individuals with cardiovascular risk factors and ictus antecedents have an increased risk of developing dementia of the Alzheimer’s type in their lifetimes [24–26]. In the last 10 years it has been demonstrated that high homocysteine concentration in plasma are also a cardiovascular risk factor for cardiovascular death as well as for ischemic cardiopathy, cerebrovascular accidents or carotid atherosclerosis. The water-soluble B vitamins, especially folate and cobalamin vitamin B12, have been shown to lower plasma tHcy [27].

Increased homocysteine concentrations may follow folate depletion due to insufficient dietary intake of the vitamin, but there is also some indication that immune activation could play a role [28]. A link seems also to exist between increased homocysteine concentration and immune activation [29]. Oxidative stress induced by immune activation could play a role in the deactivation of folate and in consequence lead to moderate hyperhomocysteinemia [30].

Since dementia of the Alzheimer’s type and vascular dementia contribute >90% of total dementias, the identification of associated risk factors are of great clinical interest. Hyperhomocysteinemia has been identified as such a factor, as well as vitamins B12 and folate supplements have been proposed as a possible treatment.

In the present study, the increased tHcy and decreased B12 and FOL observed in AD, VaD and DM groups are well in line with published results, with a highly significant difference between all groups. As increased tHcy and decreased B12 and FOL plasmatic concentrations are characteristic both of diabetes and dementias, it was expected that the superimposed effects of these conditions would result in larger differences for the AD+DM and VaD+DM groups. In fact, tHcy, B12 and FOL values in AD and VaD patients with a concomitant DM condition are closer to non-demented subjects (**Fig. 1**).

Thus, the clear-cut difference between non-diabetic demented patients and controls is masked by the DM condition resulting, as shown in **Fig. 2**, in similar shapes for the effect of DM in the demented groups and opposite to the non-demented.

The superimposed diabetic condition in demented patients results in a paradoxical effect, which can be described as a “better condition” in terms of the variables tHcy, B12 and FOL. As a risk factor for AD and VaD, tHcy might be masked by a concomitant DM condition, which not only is a clinically interesting result, but also points to possible relations between the metabolic alterations in AD, VaD and DM. While a mechanism to solve this paradox cannot be proposed yet, many studies point to existing relations between these diseases. A similar effect was reported in the antioxidant profiles of these groups of patients [18,31].

Finally, in relation with the hypotheses tested in the present study, it might be concluded that: (a) in diabetic living patients a significant association exists between the variables and the disease when compared with the controls, while these differences results much more evident in demented patients; (b) contrary to our hypothesis, the profiles of demented diabetic patients (AD+DM and VaD+DM) resulted closer to controls; (c) being intermediate between pure demented and controls, the profiles of demented diabetic patients cannot be recognized. This suggests
that the potential diagnostic value of the variables studied might be masked by the concomitant diabetic condition.

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