Homocysteine, Liver Function Derangement and Brain Atrophy in Alcoholics

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Abstract

Aims: Hyperhomocysteinemia may be involved in the development of brain atrophy in alcoholics. Its pathogenesis is multifactorial. In the present study, we analyse the relationship between homocysteine levels and brain atrophy, and the relative weight of co-existing factors such as liver function impairment, the amount of ethanol consumed, serum vitamin B12, B6, and folic acid levels on homocysteine levels and brain alterations in alcoholic patients.

Methods: We included 59 patients admitted to this hospital for major withdrawal symptoms and 24 controls. The mini-mental state examination test and a brain computed tomography (CT) scan were performed and several indices were calculated. Serum levels of homocysteine, folic acid, vitamin B6 and vitamin B12 were determined. Liver function was assessed by Child–Pugh score. The daily consumption of ethanol in grams per day and years of addiction were recorded.

Results: A total of 83.6% and 80% of the patients showed cerebellar or frontal atrophy, respectively. Patients showed altered values of brain indices, higher levels of homocysteine and vitamin B12, but lower levels of folic acid, compared with controls. Homocysteine, B12 and liver function variables showed significant correlations with brain CT indices. Multivariate analyses disclosed that Pugh’s score, albumin and bilirubin were independently related to cerebellar atrophy, frontal atrophy, cella index or ventricular index. Serum vitamin B12 was the only factor independently related to Evans index. It was also related to cella index, but after bilirubin. Homocysteine levels were independently related to ventricular index, but after bilirubin.

Conclusion: Vitamin B12 and homocysteine levels are higher among alcoholics. Liver function derangement, vitamin B12 and homocysteine are all independently related to brain atrophy, although not to cognitive alterations.

Short summary: Hyperhomocysteinemia has been described in alcoholics and may be related to brain atrophy, a reversible condition with an obscure pathogenesis. We studied 59 patients and found that liver function derangement, vitamin B12 and homocysteine levels are all independently
related to brain atrophy assessed by computed tomography, although we found no association between these parameters and cognitive alterations.

INTRODUCTION

Brain atrophy is a common finding among alcoholics (de la Monte and Kril, 2014) associated with cognitive alterations in 50–80% of alcoholic patients (Bates et al., 2002). Despite this high prevalence, its pathogenesis is poorly understood. As was already pointed out several decades ago (Butterworth, 1995), many factors, including liver function impairment, may contribute to its development. Neuroinflammation coupled with oxidative damage may constitute a final common pathway (Qin and Crews, 2012).

In the last two decades, several reports have shown an association of increased homocysteine levels and thromboembolic risk (Ray, 1998; Cattaneo, 1999). More recently, a relationship between hyperhomocysteinemia, brain atrophy and cognitive impairment in older patients (Sachdev, 2005) has been pointed out, and the importance of folate deficiency and hyperhomocysteinemia as markers of brain atrophy in elderly subjects after stroke has been underscored (Yang et al., 2007). In a community-based study, Vogiatzoglou et al. (2008) also found that low vitamin B12 is related to an increased rate of brain volume loss throughout 5 years, and to cognitive deficits, even in well-nourished, community-dwelling elderly individuals (Vogiatzoglou et al., 2013).

Hyperhomocysteinemia is also related to brain atrophy in alcoholics (Bleich et al., 2003). Homocysteine levels are increased in alcoholics mainly because acetaldehyde impairs methionine synthase activity and ethanol-induced reactive oxygen species impair the activity of methionine adenosyltransferase, blocking the formation of S-adenosylmethionine (SAM) (Kharbanda, 2009). Catabolism of homocysteine by the transsulphuration pathway is also impaired, mainly because of the fact that SAM is a necessary cofactor for cystathionine beta synthase (Fig. 1), and eventually, B6 deficiency may further decrease the activity of this enzyme. Therefore, increased homocysteine causes an increase in S-adenosyl homocysteine, which is able to enhance tumour necrosis factor alpha (TNF-α) expression (Song et al., 2004). Enhanced TNF-α expression may in turn lead to organ damage, at least in the liver. In the brain, the ability of homocysteine to activate the N-methyl-D-aspartate receptor may cause excitotoxicity and contribute to brain atrophy, at least in the hippocampus (Bleich et al., 2003). In addition to the effect of ethanol, some studies suggest that impaired liver function may alter homocysteine levels, due to the central role of the liver on methionine metabolism (Ferré et al., 2002; Remková and Remko, 2009).

Therefore, brain atrophy ensues in alcoholics, and hyperhomocysteinemia is possibly related to its development. However, several factors may cause hyperhomocysteinemia in alcoholics, and some of them, such as altered liver function, or altered availability of folate acid, vitamin B6 and vitamin B12, may also exert direct effects on brain structure and cognitive skills. The aim of the present study is to analyse the relationship between homocysteine levels and brain atrophy, and the relative weight of co-existing factors in alcoholics, such as liver function impairment, the amount of ethanol consumed, vitamin B12, B6 and folate acid levels and impaired nutrition, on homocysteine levels and brain alterations.

Fig. 1. Homocysteine metabolism. The main elements of homocysteine metabolism are shown. THF, tetrahydrofolate; MTHF, methy1tetrahydrofolate; SAM, S-adenosylmethionine; B6, vitamin B6; B12, vitamin B12.
Table 1. Some biological features of the patients included in this study

<table>
<thead>
<tr>
<th>Feature</th>
<th>Mean ± standard deviation</th>
<th>Median (interquartile range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily ethanol consumption (g)</td>
<td>186 ± 73</td>
<td>163 (120–210)</td>
</tr>
<tr>
<td>Time of addiction (years)</td>
<td>29 ± 9</td>
<td>30 (20–37)</td>
</tr>
<tr>
<td>GGT (U/l)</td>
<td>326 ± 415</td>
<td>197 (98–321)</td>
</tr>
<tr>
<td>ASAT (U/l)</td>
<td>105 ± 126</td>
<td>63 (43–119)</td>
</tr>
<tr>
<td>ALAT (U/l)</td>
<td>66 ± 94</td>
<td>41 (24–72)</td>
</tr>
<tr>
<td>Prothrombin (%)/INR</td>
<td>1.77 ± 0.84</td>
<td>1.60 (1.20–2.15)</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>112.78 ± 100.65</td>
<td>100.90 (94.6–108.20)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.54 ± 0.77</td>
<td>3.70 (2.80–4.10)</td>
</tr>
<tr>
<td>Prothrombin activity (%)</td>
<td>74.31 ± 22.68</td>
<td>72.30 (53.60–100)</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>4.02 ± 5.59</td>
<td>1.60 (0.60–4.00)</td>
</tr>
<tr>
<td>Pugh</td>
<td>7.41 ± 2.60</td>
<td>6 (5–10)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52.54 ± 9.30</td>
<td>53 (47–60)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.55 ± 5.87</td>
<td>25.88 (23.46–31.10)</td>
</tr>
</tbody>
</table>

PATIENTS AND METHODS

Patients

As shown in Table 1, we included 59 adult, heavy alcohol-dependent patients that fulfilled the DSM-IV criteria for alcohol dependence, admitted to the Internal Medicine unit of our Hospital (via the emergency room) due to major manifestations of alcohol withdrawal syndrome, including tremor, visual, auditory and tactile hallucinations, hyperactivity, disorientation and intense diaphoresis. Patients with any other drug addiction (besides tobacco and alcohol) were excluded. The sample was composed of 51 men and 8 women, aged 52.54 ± 9.30 years. All patients underwent complete clinical and laboratory evaluation derived from blood samples obtained within 48 hours after admission (Table 2), and a brain computed tomography (CT) using a Toshiba-Aquilion 64 CT scanner (field of view 310; 100 kv, 120 mA) was performed on all of them at admission because of traumatic head injury and/or seizure. Several parameters were measured in the CT image, as reported elsewhere (García-Valdecasas-Campeol et al., 2007) and summarized below, in all but four individuals.

Methods

(a) Evaluation of brain atrophy

The following CT variables were calculated (Amodio et al., 2003):

Bifrontal index = Maximum width of the anterior horns of the lateral ventricles (HLV) in relation to the inner skull width at the same level.

Bicaudate index = Minimum width of the lateral ventricles (MLV) in relation to the inner skull at the same level.

Cella media index = Width of maximum external diameter of lateral ventricles at cella media (i.e., central part of lateral ventricles) in relation to the inner skull at the same level, which corresponds to the maximum inner skull diameter (MISD).

Evan’s index = HLV/MISD.

Ventricular index = MLV/HLV.

Huckman’s index = Bifrontal + bicaudate indices.

Table 2. The Child–Pugh prognostic score

<table>
<thead>
<tr>
<th>Clinical criteria</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum albumin &gt;3.5 g/dl</td>
<td>1</td>
</tr>
<tr>
<td>2.8–3.5 g/dl</td>
<td>2</td>
</tr>
<tr>
<td>&lt;2.8 g/dl</td>
<td>3</td>
</tr>
<tr>
<td>Prothrombin(%)/INR &gt;50%/&lt;1.7</td>
<td>1</td>
</tr>
<tr>
<td>30–50%/1.7–2.3</td>
<td>2</td>
</tr>
<tr>
<td>&lt;30%/&gt;2.3</td>
<td>3</td>
</tr>
<tr>
<td>Serum bilirubin &lt;2 mg/dl</td>
<td>1</td>
</tr>
<tr>
<td>2–3 mg/dl</td>
<td>2</td>
</tr>
<tr>
<td>&gt;3 mg/dl</td>
<td>3</td>
</tr>
<tr>
<td>Ascites</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>Medical control</td>
<td>2</td>
</tr>
<tr>
<td>Refractory</td>
<td>3</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
</tr>
<tr>
<td>Sporadic, easy to control</td>
<td>2</td>
</tr>
<tr>
<td>Poorly controlled</td>
<td>3</td>
</tr>
</tbody>
</table>

In addition, an expert neuroradiologist classified the patients according to CT scan into those showing cerebellar atrophy or not, or frontal atrophy or not.

(b) Evaluation of cognitive skills

An assessment of cognitive function [mini-mental state examination (MMSE), Folstein et al., 1975] was also performed when patients had already recovered from the withdrawal syndrome, just the day before hospital discharge.

(c) A complete history and clinical examination were obtained for each patient, including weight and height, and detailed information about drinking habit, amount of ethanol consumed and duration of addiction and the presence of ascites or encephalopathy. Based on the presence of ascites, encephalopathy, serum albumin and bilirubin levels and prothrombin activity, we classified the patients according to the Child-Pugh scoring system (Pugh et al., 1973, Table 2). This score was also calculated for patients in whom the features found on ultrasonography did not support the diagnosis of cirrhosis. We also recorded the presence or absence of malnutrition according to an already reported, validated scoring system (Santolaria et al., 2000). Tobacco smoking was also recorded, and the number of packs (20 cigarettes) smoked daily times the number of years the patient smoked was calculated (smoking pack-year index).

(d) At admission, as a part of the laboratory evaluation, we determined serum levels of homocysteine, vitamin B12, vitamin B6 and folic acid. Homocysteine levels were analysed by polarized fluorescence immunoassay (Abbott IMx system, Abbott Park, IL, USA). Serum B12 was determined by microparticle enzyme immunoassay (Abbott AxSYM system, Abbott Park, IL, USA); serum folate levels were determined by ionic capture immunoassay (Abbott AxSYM system). Plasma B6 was determined by immunoassay. These variables were also determined in 24 hospital workers, with a similar age (48.21 ± 9.40 years) and sex proportion (19 men and 5 women), drinkers of <10 g ethanol/day, who served as controls. In addition, brain CT was also

...
performed in 15 of these hospital workers (5 women, $X^2 = 2.19; P = 0.14$), aged 45.40 ± 15.21 years ($T = 1.73; P = 0.10$).

All patients gave their informed consent to enter the study that was performed following the ethical guidelines of the 1975 declaration of Helsinki and was approved by the ethical committee of our hospital (number = 2012-01).

Statistics

The Kolmogorov–Smirnov test was used to analyse if CT scan variables, B12, B6, folic acid levels and homocysteine followed a normal distribution or not. In those cases in which variables did not follow a normal distribution, we used non-parametric tests, such as Mann–Whitney’s and Kruskal–Wallis tests (KW) to assess differences between groups, and Spearman’s correlation ($\rho$). On the other hand, Student’s t-test, variance analysis with Student–Newman–Keuls post-hoc analysis and Pearson’s correlation were used to analyse parametric variables, and $\chi^2$ was used to study the association between two qualitative variables. We performed stepwise logistic regression and/or multiple correlation analyses between each of the variables derived from CT measurements and MMSE test and all the other variables analysed, to disclose which of them were independently related to brain morphological and/or functional alterations. Statistical analyses were performed using SPSS software (Chicago, IL, USA).

RESULTS

Table 3. Means and standard deviations of the different brain indices measured in patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 15)</th>
<th>Patients (N = 55)</th>
<th>Z; P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evans index</td>
<td>0.2498 ± 0.0587</td>
<td>0.2905 ± 0.0466</td>
<td>2.677; 0.007</td>
</tr>
<tr>
<td>Bicaudate index</td>
<td>0.1335 ± 0.0285</td>
<td>0.1789 ± 0.0326</td>
<td>4.182; &lt;0.001</td>
</tr>
<tr>
<td>Cella index</td>
<td>0.1828 ± 0.0275</td>
<td>0.2939 ± 0.0878</td>
<td>4.253; &lt;0.001</td>
</tr>
<tr>
<td>Bifrontal index</td>
<td>0.2831 ± 0.0581</td>
<td>0.3355 ± 0.0593</td>
<td>3.071; 0.002</td>
</tr>
<tr>
<td>Ventricular index</td>
<td>0.5097 ± 0.08205</td>
<td>0.5549 ± 0.1259</td>
<td>1.761; 0.078 (NS)</td>
</tr>
<tr>
<td>Huckman’s index</td>
<td>0.4160 ± 0.0794</td>
<td>0.5144 ± 0.0826</td>
<td>3.571; &lt;0.001</td>
</tr>
</tbody>
</table>

Table 4. Mean values of homocysteine and vitamins among patients and controls

|                                | Patients (N = 59) | Controls (N = 24) | Z (|t|); P       |
|--------------------------------|------------------|------------------|-----------|
| Homocysteine (µg/dl)            | 15.03 ± 7.01     | 11.68 ± 2.40     | 2.06; 0.039 |
| Vitamin B12 (pg/ml)             | 999.34 ± 616.75  | 382.13 ± 127.62  | 4.87; <0.001 |
| Vitamin B6 (nmol/l)             | 110.59 ± 115.04  | 46.69 ± 18.59    | 1.70; NS    |
| Folic acid (nmol/l)             | 6.20 ± 5.42      | 7.20 ± 3.12      | 2.32; 0.021 |
| Age (years)                     | 52.54 ± 9.30     | 48.29 ± 9.30     | 1.88; NS    |

Relationship between vitamins involved in homocysteine metabolism and brain atrophy

Homocysteine was directly related to cella index ($\rho = 0.43; P < 0.001$), bicaudate index ($\rho = 0.29; P = 0.033$) and ventricular index ($\rho = 0.32; P = 0.017$). When cella index was compared among terciles of homocysteine, those belonging to the highest tercile showed the most dilated index value (KW = 13.78; P < 0.001). No association was observed between homocysteine and cerebellar or frontal atrophy. Homocysteine was associated with age ($R = 0.34; P = 0.009$).

Vitamin B12 was inversely related to ventricular index ($\rho = -0.31; P = 0.021$) and, inversely, to homocysteine ($\rho = -0.28; P = 0.012$). When terciles of vitamin B12 were compared with Evans index, those belonging to the lowest or highest tercile showed significantly more diluted index value (KW = 9.16; P = 0.01, Fig. 2A). A similar trend, although non-significant, was observed when vitamin B12 terciles were compared with bifrontal index (Fig. 2B; KW = 4.86; P = 0.088), and Huckman’s index (Fig. 2C; KW = 5.05; P = 0.08). There was a trend to higher vitamin B12 values among those with cerebellar atrophy (Z = 1.89; P = 0.059), but no association at all was observed with the presence of frontal atrophy.

Vitamin B6 was inversely related to vitamin B12 ($\rho = -0.33; P = 0.012$). Patients with cerebellar atrophy showed lower vitamin B6 values than patients without atrophy (Z = 2.28; P = 0.023), but no association was observed between vitamin B6 levels and the presence or not of frontal atrophy. Folic acid was not related to any of the aforementioned parameters.

Relationship between vitamins involved in homocysteine metabolism and liver function

Both vitamin B12 and vitamin B6, but not homocysteine or folic acid, showed a close relationship with liver function. Indeed, serum vitamin B12 was closely, inversely related to albumin ($\rho = -0.46$), prothrombin ($\rho = -0.67$), and directly to bilirubin ($\rho = 0.69$) and Pugh’s score ($\rho = 0.63; P < 0.001$ in all the cases). Vitamin B6 was significantly related to albumin ($\rho = 0.33; P = 0.01$) and prothrombin ($\rho = 0.26; P = 0.045$) and, inversely, to bilirubin ($\rho = -0.37; P = 0.004$) and Pugh’s score ($\rho = -0.36$;
Therefore, vitamin B12 levels increased in patients with a more deranged liver function, whereas vitamin B6 levels showed an opposite trend.

In contrast, no relationship was found between serum levels of homocysteine, vitamin B6, B12 and folic acid and serum creatinine.

Relationship between vitamins involved in homocysteine metabolism and nutritional status, tobacco and ethanol consumption and hypertension

An inverse correlation was observed between pack-year index and vitamin B12 levels (\( \rho = -0.39, P = 0.026 \)). Also, a weak, significant correlation was observed between serum gamma-glutamyltranspeptidase (GGT) and vitamin B12 levels (\( \rho = 0.26, P = 0.049 \)), whereas homocysteine was also related to mean corpuscular volume (MVC) (\( \rho = 0.34, P = 0.009 \)). No associations were observed between vitamin levels and homocysteine and arterial pressure, body mass index (BMI), daily amount of ethanol ingestion or drinking duration. Also, no relationship was observed between levels of these vitamins and subjective nutritional status assessment.

Liver function and brain atrophy: multivariate analysis

We failed to find any association between the presence of cirrhosis and the parameters related to ventricular dilatation. However, patients with the worst liver function, assessed by Child score, showed the most dilated values for Huckman’s index (KW = 9.39; \( P = 0.009 \)), bicaudate index (KW = 9.23; \( P = 0.01 \)), Evans index (KW = 10.10; \( P = 0.006 \)) and bifrontal index (KW = 10.03; \( P = 0.007 \)). Patients with cerebellar atrophy showed higher Pugh’s score (\( Z = 2.23; P = 0.026 \)) and bilirubin levels (\( Z = 2.38; P = 0.017 \)). Patients with frontal atrophy showed lower albumin levels (\( Z = 2.50; P = 0.013 \)) and a higher Pugh’s score (\( Z = 1.94; P = 0.052 \)).

No association was found between these brain indices and the following parameters: prothrombin, albumin, bilirubin; daily amount of ethanol intake and drinking duration; systolic and diastolic blood pressure; BMI and nutritional status assessed by the subjective scoring system; and mini-mental test score.

Age was related to Evans (\( \rho = 0.34; P = 0.011 \)), bicaudate (\( \rho = 0.38; P = 0.004 \)), Huckman’s (\( \rho = 0.32, P = 0.02 \)), cella (\( \rho = 0.28; P = 0.035 \)) and bifrontal index (\( \rho = 0.27; P = 0.047 \)), but it was not associated with mini-mental score, or the presence of either cerebellar or frontal atrophy.

We performed several multivariate analyses, stepwisely introducing the variables age, Pugh’s score, albumin, bilirubin, prothrombin activity and serum levels of vitamin B12, vitamin B6, homocysteine and folic acid, to discern which of these variables were independently related to each of the analysed CT indices or the presence of cerebellar or frontal atrophy. Variables related to liver function, such as Pugh’s score, albumin and bilirubin, are independently related to cerebellar atrophy, frontal atrophy, cella index or ventricular index. Age was the only variable that showed an independent association with bicaudate index and Huckman’s index. Serum vitamin B12 was the only factor independently related to Evans index, and it was also related to cella index, but after bilirubin. Finally homocysteine levels were independently related to ventricular index, but after bilirubin.

No variable was selected as an independent factor related to bifrontal index or MMSE test.
DISCUSSION

In this study, it was shown that some vitamins, such as B12 and B6 and homocysteine, are clearly related to several parameters associated with brain atrophy. Brain atrophy in alcoholics is a common finding, although its pathogenesis is quite far from being clear. Undoubtedly, oxidative damage seems to play a role, as pointed out by several authors (Davis and Syapin, 2004; Crews and Nixon, 2009; Qin et al., 2013; Alfonso-Loeches et al., 2014), due to the direct effects of ethanol and/or increased cytokine secretion. Indeed, ethanol abstinence markedly improves alcoholism-associated brain atrophy (Demirakca et al., 2011). In the present study, however, no relation was observed between the amount of ethanol intake and brain atrophy. One reason for this finding may rely on the fact that we included patients who were heavy drinkers (with an intake of nearly 200 g of ethanol per day during more than 20 years), which may have obscured a possible association due to a ‘saturation effect’. On the other hand, we specifically excluded from the study alcoholic consumers of other drugs (besides tobacco). In part, this is a strength of this study (as well as the relatively young age of the included patients), because older age and the consumption of other illicit drugs may also cause brain atrophy and impair cognitive skills, and we here analyse the sole effects of ethanol. But it can be also viewed as a limitation, since this addictive pattern (only to alcohol and to the widespread smoking habit), although very common in the rural areas of our geographical environment, may differ somewhat from that observed in urban areas and large cities, limiting the application of our results to other cohorts of alcoholics. Another limitation of this study is that we did not measure blood alcohol levels at admission. In a recent study, it was reported that homocysteine levels on day 1 of alcohol withdrawal are significantly influenced by blood ethanol levels on admission (Heese et al., 2012).

Lacking blood ethanol data, we cannot discern the importance of the effect of ethanol concentration on homocysteine levels in this study.

An interesting finding is the relationship observed between severity of liver dysfunction and brain alterations observed in the alcoholics. Several mechanisms have been proposed to explain this association. First, patients with liver cirrhosis have generally been drinking for a longer time and in greater amounts than patients without cirrhosis (Kamper-Jørgensen et al., 2004), although this is not a universally valid assertion (Bellantoni et al., 1997). It has also been proposed that repeated episodes of subclinical encephalopathy could lead in time to some degree of atrophy, given that brain atrophy was also observed in non-alcoholic cirrhotics with repeated episodes of encephalopathy (Zeneroli et al., 1987). Recently, the term liver–brain axis has been coined; in liver steatosis toxic lipids are = body mass index
CT = computed tomography
GGT = gamma-glutamyl-transpeptidase
HLV = maximum width of the anterior horns of the lateral ventricles
KW = Kruskal–Wallis test
MCV = mean corpuscular volume
MISD = maximum inner skull diameter
MLV = minimum width of the lateral ventricles
MMSE = mini-mental state examination
NMDA = N-methyl-D-aspartate
ROS = reactive oxygen species
SAM = S-adenosylmethionine
SPSS = Statistical Package for Social Sciences
TNF = tumour necrosis factor
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