Research Article

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Cerebral magnetic resonance imaging reveals marked abnormalities of brain tissue density in patients with cirrhosis without overt hepatic encephalopathy

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Background & Aims: We applied advanced magnetic resonance imaging and Voxed based Morphometry analysis to assess brain tissue density in patients with cirrhosis.

Methods: Forty eight patients with cirrhosis without overt hepatic encephalopathy (17 Child A, 13 Child B, and 18 Child C) and 51 healthy subjects were matched for age and sex. Seventeen patients had history of overt hepatic encephalopathy, eight of them had minimal hepatic encephalopathy at inclusion, 10 other patients had minimal hepatic encephalopathy at inclusion but without history of previous overt hepatic encephalopathy, and 21 patients had none of these features.

Results: Patients with cirrhosis presented decreased brain density in many areas of the grey and white matter. The extension and size of the affected areas were greater in patients with alcoholic cirrhosis than in those with post-hepatitic cirrhosis and correlated directly with the degree of liver failure and cerebral dysfunction (as estimated by neuropsychological tests and the antecedent of overt hepatic encephalopathy). Twelve additional patients with cirrhosis who underwent liver transplantation were explored after a median time of 11 months (7–50 months) after liver transplant. At the time of liver transplantation, three patients belonged to class A of the Child-Pugh classification, five to class B and four to class C. Compared to healthy subjects, liver transplant patients showed areas of reduced brain density in both grey and white matter.

Conclusions: These results indicate that loss of brain tissue density is common in cirrhosis, progresses during the course of the disease, is greater in patients with history of hepatic encephalopathy, and persists after liver transplantation. The significance, physiopathology, and clinical relevance of this abnormality cannot be ascertained from the current study.

Keywords: Cirrhosis; Hepatic encephalopathy; Magnetic resonance images; Voxel-based Morphometry.

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Introduction

Hepatic encephalopathy is a cerebral disorder of patients with liver failure and/or porto-systemic shunting caused by ammonia and other endogenous substances that escape from hepatic metabolism [1]. The spectrum of the syndrome ranges from minimal hepatic encephalopathy to deep coma. Since hepatic encephalopathy reverses in most patients after appropriate treatment, it is considered a functional disorder [2]. The methodology used to explore cerebral function in cirrhosis includes: the EEG and the mean dominant frequency, neuropsychological tests exploring attention and cognitive functions, and the critical flicker frequency and other recently introduced computerized tests that estimate the reactive ability of the patients to a visual stimulus [3–6]. Most of these tests have been developed to diagnose minimal hepatic encephalopathy.

Magnetic resonance imaging is widely used for research and diagnosis of cerebral diseases. Voxel-based Morphometry, which measures brain tissue density (concentration), has been proved of great interest in the assessment of regional areas of atrophy in neurodegenerative cerebral diseases such as Alzheimer's Disease, Huntington's Chorea, and Multiple Sclerosis [7–11]. Regional areas of density loss diagnosed by Voxel-Based Morphometry in Alzheimer Disease correlate with areas of atrophy on histopathology [12].

The current article reports the first study using Voxel-based Morphometry in a large series of patients with cirrhosis of different aetiologies, with and without previous history of hepatic encephalopathy but without overt hepatic encephalopathy at the time of the investigation. The aim of the study was to assess brain tissue density in patients with cirrhosis and its relationship with the severity of the disease and history of hepatic encephalopathy.

Patients and methods

Forty-eight patients with compensated and decompensated cirrhosis and 51 healthy subjects of similar age and educational level were investigated. No subject with active alcoholism (three months prior to the study), gastrointestinal haemorrhage or bacterial infection (1 month prior to the study), age lower than 18 or greater than 75 years, neurological or psychiatric diseases, overt hepatic encephalopathy, transjugular intrahepatic portosystemic shunt or surgical portocaval shunt, treatment with drugs that could alter cerebral function or contraindication to magnetic resonance imaging (MRI) were studied. The study was approved by the Ethical Committee of the Hospital Clinic of Barcelona. Written informed consent was obtained from each subject.

Twelve additional cirrhotic patients submitted to liver transplantation were studied after a median period of 11 months (range 7–50 months) after liver transplant and compared to 12 healthy subjects matched by sex and age. Patients with liver transplant had minimal or no fibrosis in liver biopsy at the time of the study.

Physical examination, standard laboratory parameters, and a battery of neuropsychological tests were performed in all subjects. Patients with ascites were studied after at least 1 week without diuretics. Neuropsychological tests were administered by a trained neuropsychologist and included NCT-A, NCT-B, block-design test, and digit-symbol test. Minimal hepatic encephalopathy was defined as the presence of at least two neuropsychological tests with abnormal values (± 2SD of values obtained in normal subjects) [13]. History of overt hepatic encephalopathy was retrospectively taken from the clinical records. In our Unit, we use the West Haven criteria to classify hepatic encephalopathy.

Magnetic resonance imaging

3D-Inversion Recovery Prep images of the entire brain (TR = 12 ms, TE = 52 ms, FOV = 24, acquisition matrix 256 × 192 mm, slice thickness 1.5 mm) were obtained using a 1.5T GE Nvi/Cvi Magnetic Resonance Apparatus and the head coil. Regular quality assurance was performed during each study by an experienced observer, and only good quality images were accepted.

Voxel Based-Morphometry (VBM) allows comparing local concentrations of grey and white matter between two groups of subjects. This is performed at a voxel level, which in this study had a dimension of 1 mm^3, and involves spatial normalization of all MR images to the same stereotactic space, followed by segmenting the tissue from the normalized images, smoothing, and performing a statistical analysis. At the end, a statistical parametric map is obtained showing regions where a concentration of a certain tissue type differs between groups. In our study, MR images were post processed using methods implemented in the statistical parametric mapping software (SPM2, Welcome Department of Cognitive Neurology, London, UK). Smoothed grey and white matters were analysed with an ANCOVA model to detect regions of decreased density and two-side *t*-test were used to assess differences in grey or white matter density between groups. Age and sex were included as nuisance covariates. False Discovery Rate (FDR) was set at $p < 0.05$ for corrected VBM. When patients and normal subjects are compared, a one way statistical analysis is performed, subtracting from the voxel-density detected in controls the density detected in patients in a voxel by voxel manner. However, when two groups of patients are compared, the analysis has to be performed in two ways to assess areas with low density in one group with respect to the other and vice versa. The *Z* scores are a way that SPM uses to display and analyze the *p* values from the *t* statistics. They are the numbers from the unit normal distribution that would give the same *p* values as the *t* statistics. The areas (clusters) with the higher *Z* scores indicate the most robust changes in brain density. Clusters with a size lower than 100 k were not considered for statistical comparisons. For further details on post processing analysis and other aspects of the methodology used see references [7,14,15].

Statistical analysis of clinical and biochemical data

The non-parametric Mann–Whitney test for continuous data and the Chi-square and Fischer tests for categorical data were applied for comparisons between clinical and laboratory data. The SPSS 12 statistical software (SPSS Inc. and Microsoft Corp., Chicago, IL) was used and results are given as mean ± SD. $p < 0.05$ was considered statistically significant.

Results

Clinical and laboratory parameters of the group of patients with cirrhosis are shown in Table 1. Seventeen patients had previous episodes of overt hepatic encephalopathy and 18 had minimal

Table 1. Demographic, clinical, and biochemical data of the patients with cirrhosis and healthy subjects included in the study.

Variable	Cirrhotic patients (n = 48)	Healthy subjects (n = 51)	*p* value
Age (years)*	61 ± 9 (44-75)	60 ± 11 (33-85)	NS
Sex: male/female (n)	30/18	23/28	NS
Years of education*	9 ± 5 (0-20)	10 ± 4 (3-20)	NS
Post-hepatitic cirrhosis (n)	27	-	
Alcoholic cirrhosis (n)	21	-	
Previous hepatic encephalopathy (n)	17	-	
Ascites at inclusion (n)	30	-	
Child-Pugh's class: A/B/C (n)	17/13/18	-	
Child-Pugh's score*	8 ± 2.5 (5-13)	-	
MELD score	13 ± 5 (7-27)	-	
Serum bilirubin (mg/dl)*	3.1 ± 4.6 (0.4-30.5)	0.6 ± 0.3 (0.3-1.7)	<0.001
Prothrombin time (%)*	62 ± 17 (20-100)	95 ± 6 (82-100)	<0.001
Serum albumin (g/L)*	32 ± 7 (21-47)	42 ± 3 (35-48)	<0.001
Serum creatinine (mg/dl)*	1.05 ± 0.4 (0.7-2.8)	0.95 ± 0.15 (0.7-1.3)	NS
BUN (mg/dl)*	23 ± 14 (7-68)	19 ± 4.6 (9-26)	NS
Serum sodium (mEq/L)*	134 ± 7 (118-147)	140 ± 2 (136-144)	<0.001
Serum ammonia (μMol/L)*	47 ± 28 (7-129)	18 ± 11 (0-47)	<0.001
Hematocrit (%)*	35 ± 7 (20-49)	40 ± 4 (34-50)	<0.001
Minimal hepatic encephalopathy (n)	18	-	

*Values are mean ± SD or number of patients. Values in brackets are ranges.