ALCOHOLISM CLINICAL & EXPERIMENTAL RESEARCH

Relationship Between Liver Function and Brain Shrinkage in Patients with Alcohol Dependence

Chun-Hsin Chen, Jonathan Walker, Reza Momenan, Robert Rawlings, Markus Heilig, and Daniel W. Hommer

Background: Oxidative stress has been proposed as one of the mechanisms of alcohol-induced brain shrinkage and alcohol-induced hepatotoxicity. The aim of this study was to assess the correlations between liver function and brain volume (BV) measurements in patients with alcohol dependence.

Methods: We recruited 124 patients with alcohol dependence and 111 healthy control subjects from National Institute of Health, National Institute on Alcohol Abuse and Alcoholism inpatient alcohol treatment program. Gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), as well as hematocrit (Hct) and albumin were assayed shortly after admission. Magnetic resonance imaging examination was conducted in both groups (after 3-week abstinence in the patient group). We used stepwise linear regression analyses to determine the variables most strongly correlated with brain shrinkage.

Results: Patients with alcohol dependence had lower BV, and greater brain shrinkage as measured by gray matter ratio (GMR), white matter ratio (WMR), brain ratio (BR), and higher cerebrospinal fluid ratio ratio (CSFR) compared with their healthy counterparts. Age and sex were significantly correlated with some BV measurements in both patient and control groups. Body mass index (BMI) was significantly correlated with CSFR, BR, GMR, and WMR; Hct with CSFR and BR; serum GGT level with BV, CSFR, BR, GMR, and WMF in the patient group. No biological variables were correlated with BV indices in the control group. In gender-stratified analysis, age was significantly correlated with brain shrinkage in male patients but not in female patients. Serum GGT level in male and female patients, Hct in male patients, and AST levels in female patients were significantly correlated with brain shrinkage.

Conclusions: Our results showed that the higher levels of liver function indices, especially GGT, correlated with BV shrinkage as measured using CSFR, BR, GMR, and WMR in patients with alcohol dependence but not in controls. Serum GGT level outweighed aging effect on brain shrinkage in female patients.

Key Words: Alcohol Dependence, MRI, Liver Function, Brain Shrinkage.

STRUCTURAL BRAIN ALTERATIONS, such as reduction in white matter, gray matter, and overall brain shrinkage, have been documented in patients with alcohol dependence (Fein et al., 2002; Jernigan et al., 1991; Pfefferbaum et al., 1992). These changes are more pronounced in female patients with alcohol dependence (Hommer et al., 2001; Mann et al., 2005); though, some did not find a gender difference (Pfefferbaum et al., 2001). However, the causes of these brain volumetric changes in patients with alcohol dependence are not clearly understood. One possible mechanism is alcohol-induced neurodegeneration caused by oxidative stress (Crews and Nixon, 2009). Oxidative stress may affect not only the central nervous system, but also the process of alcohol-induced liver injury (Fataccioli et al., 1999; Kono et al., 2000).

The metabolism of alcohol in the context of chronic alcohol consumption results in excessive reactive oxygen species (ROS) production, which results in excessive oxidative stress (De Minicis and Brenner, 2008). These ROS products cause DNA damage, generate protein adducts, and initiate lipid peroxidation. There are several antioxidant defense mechanisms that are involved in the elimination of ROS, including superoxide dismutase (SOD) and glutathione peroxidase (GPX). Decreased SOD and GPX activities, and increased serum level of malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG), which are products of lipid peroxidation and oxidative DNA damage, have been shown

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during early alcohol-withdrawal period in patients with alcohol dependence (Chen et al., 2011; Huang et al., 2009). Although a role of oxidative stress related to alcohol-induced neurotoxicity has been suggested by several studies (for review of these studies, see Crews and Nixon, 2009), there has been no evidence that oxidative stress correlated with alcohol-induced brain damage in patients with alcohol dependence. However, a recent study found that functional SOD2 gene polymorphisms influenced gray matter loss in patients with alcohol dependence, suggesting that oxidative stress may be one of the mechanisms for alcohol-induced neurotoxicity, but this study did not directly measure any oxidative stress markers (Srivastava et al., 2010).

Oxidative stress has been proposed to be associated with alcohol-induced liver injury (Fataccioli et al., 1999; Kono et al., 2000). Although several markers, such as MDA and 8-OHdG (Mayne, 2003), have been used to measure oxidative stress, these biomarkers are not routinely measured in clinical practice. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT) are serum markers used to assess liver function that have been used as surrogate markers of excessive alcohol consumption (Conigrave et al., 2003) and have been shown to be significantly correlated with serum MDA level in patients with alcohol dependence (Huang et al., 2009). In addition, GGT has also been shown to be associated with lipid peroxidation in hepatocytes (Paolicchi et al., 1997). Although liver function indices have been shown to be associated with brain shrinkage as measured by computed tomography (Junghanns et al., 2004; Mutzell, 1992) in patients with alcohol dependence, using categorical grouping in Mutzell's study and not controlling age and drinking characteristics in Junghanns's study might limit the interpretation of these results. Pfefferbaum and colleagues (2004) have found high, but not significant, correlations between serum GGT level and cortical white matter ($r = -0.48$) and third ventricle size ($r = 0.42$) in patients with alcohol dependence. We suggest the sample size (only 16 GGT data available) limited the power to detect significant correlations between GGT level and brain volume (BV) measurements in Pfefferbaum and colleagues' (2004) study. Therefore, the aim of this study was to ascertain the relationships between liver function and brain shrinkage taking potential confounding factors into consideration in a larger sample of patients with alcohol dependence.

MATERIALS AND METHODS

The study was reviewed and approved by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) Institutional Review Board before recruiting patients. All participants provided written informed consent to participate after the study procedures were explained to them.

Subjects

A total of 124 patients with alcohol dependence (79 males and 45 females) and 111 healthy control subjects (73 males and 38 females) were recruited. All patients were admitted to the NIAAA inpatient unit at the National Institutes of Health (NIH) Clinical Center in Bethesda, Maryland, for an inpatient alcoholism treatment program. Healthy control subjects with no history of significant medical illness or psychiatric disorders were recruited through the Normal Subject Office of the Clinical Center of the NIH.

All patients met Diagnostic and Statistical Manual, Fourth Edition (DSM-IV) criteria for alcohol dependence. Exclusion criteria for patients with alcohol dependence included the following conditions: met the criteria for alcohol abuse but not alcohol dependence; had a history of delirium tremens or psychotic disorders; had an IQ score < 80; demonstrated signs of Wernicke's encephalopathy, dementia, or Korsakoff's disease; had a history of intravenous drug use at any time during their life or substance dependence other than alcohol or nicotine in the 6 months preceding admission. No subjects were thiamine deficient at admission. The comparison subjects had no disorders meeting DSM-IV criteria for psychotic disorder or substance-use disorders, except nicotine, and reported no first-degree relatives with a history of alcohol dependence or the problem of drinking. None of the control subjects reported drinking more than 3 drinks a day on a regular basis.

Clinical Assessment

Based on history, physical examination, blood chemistry, and a negative urinary drug screen, all subjects were judged to be medically healthy at the time of the scan. None of the patients with alcohol dependence showed signs of clinically significant hepatitis or cirrhosis, or any neurological disorders associated with alcoholism, or had a history of head injury requiring hospitalization. The patients with alcohol dependence were scanned about 3 weeks after admission or, in cases of patients who had been directly transferred from another hospital, at least 3 weeks after their last alcohol use. The mean time between their last drink and magnetic resonance imaging (MRI) scan was 22.2 (SD = 5.2) days.

A social worker administered a semi-structured lifetime drinking history interview to each subject. Alcohol-use history was divided into epochs of various use patterns according to each respondent's history, and from these epochs, we calculated 4 drinking history parameters: (i) age at onset of heavy drinking, defined a priori as the age at which the subject reported first consuming the equivalent of 90 drinks in a 1-month period; (ii) years of heavy drinking, defined as the cumulative total contiguous or noncontiguous months during which the subject drank 90 drinks per month (it is noted that because subjects often maintain this high level of alcohol use for at least 12 consecutive months, months are summed into years); (iii) estimated lifetime alcohol consumption (kg of ethanol), which is a summation of all alcohol ingestion, including during periods where ingestion did not reach 90 drinks per month; and (iv) drinking quantity in the last 180 days (kg of ethanol), defined as a summation of all alcohol ingestion in the last 180 days.

Magnetic Resonance Imaging, Scan Acquisition, and Analysis

Subjects underwent imaging with 1.5-T MRI (GE Medical Systems, Milwaukee, WI) that used a fast spoiled gradient/recall acquisition in the steady-state sequence. We used a gapless series of high-contrast, 2-mm thick, T1-weighted coronal images (repetition time = 25 ms, inversion time = 5 ms, and echo time = 16 ms). The images were acquired with a 256×256 matrix and a 240×240-mm field of view. Each volumetric brain consisted of 124 coronal slices with voxel size of $0.9375 \times 0.9375 \times 2.0$ mm. The intracranial tissue was separated from the skull on coronal sections by using a hand-driven cursor. The intracranial volume (ICV) included the cerebrum and cerebrospinal fluid (CSF) spaces covering the cortex but excluded the cerebellum and CSF of the posterior fossa. ICV was automatically segmented into CSF volume (CSFV), gray matter volume (GMV), and white matter volume (WMV) according to the

methods previously described and validated (Momenan et al., 1997) that used voxel intensity to perform a K-means clustering procedure. BV was calculated by adding GMV and WMV.

We chose to include both ICV and BV in our analyses because they are conceptually distinct. The skull (and thus ICV) grows in tandem with the brain during childhood until about age 15, at which point the BV (but not ICV) begins to shrink in late adolescence and throughout adulthood (Courchesne et al., 2000). Therefore, ICV in an adult is a trait characteristic that reflects maximal brain growth during development, but BV in adults represents the outcome of both growth and atrophy processes. As ICV is a function of the *sum* of maximum CSFV, GMV, and WMV, we used a ratio approach to avoid the confounding effect of ICV. Ratios were created between a volume of interest and the remainder of the ICV (ICV minus the volume of interest), that is, CSF ratio (CSFR) = CSFV/(ICV − CSFV); brain ratio (BR) = BV/(ICV − BV); gray matter ratio (GMR) = GMV/(ICV − GMV); and white matter ratio (WMR) = WMV/(ICV − WMV). This method of calculating ratios has been previously suggested as an appropriate treatment for compositional data (McCrory and Ford, 1991).

Laboratory Assays

A venous blood sample was collected in the morning after an overnight fasting for both patient and control groups. The collection time for the patient group was at the first morning after patients' admission. The median time of the interval between blood withdrawal and last drink was 1 day (range from 0 to 14 days). Serum levels of albumin, ALT, AST, GGT, hematocrit (Hct), and mean corpuscular volume of red blood cells (MCV) were measured using automated systems at NIH Clinical Center, Bethesda.

Statistical Analysis

We used descriptive statistics to show the demographic and clinical characteristics. As the biological markers, including serum ALT, AST, and GGT levels, were not normally distributed (Kolmogorov–Smirnov test), we log-transformed these variables to normalize their distribution. The main demographic and clinical variables were compared between groups using the Student's *t*-test for continuous variables and the chi-square test for categorical variables. We used Pearson's correlation to test the relationship between BV indices and various demographic, clinical, and biological variables. If there were any variables significantly correlated with any index of BV in Pearson's correlation analysis, we used them as independent variables in a following forward stepwise linear regression analysis. The dependent variables were CSFR, BR, GMR, and WMR. Because of multiple comparisons in BV measures, we set p-value < 0.01 as significant in our analyses.

RESULTS

Table 1 shows the demographic, clinical, and biological characteristics of recruited patients with alcohol dependence and healthy control subjects. Patients with alcohol dependence were older, had fewer years of education, longer duration of smoking, greater alcohol consumption, and higher levels of liver enzymes and MCV values. Hct and serum albumin concentrations did not significantly differ between the patients with alcohol dependence and the controls (Table 1). Abnormal liver functions, defined by NIH Clinical Laboratory, are as follows: AST higher than 34 U/l, ALT higher than 41 U/l, and GGT higher than 85 U/l. A significantly higher portion of the individuals in the patient group had elevated serum liver enzymes indicating compromised liver function (AST: 59.7% in the patient group vs. 7.2% in the control group; ALT: 46.8% vs. 6.8%; GGT: 52.4% vs. 0%, respectively; chi-square test, all $p < 0.001$).

Table 2 shows the brain volumetric data in the patient and control groups. Patients with alcohol dependence had lower BV, GMR, WMR, and BR, and higher CSFR. Because the patient group was older and had lower educational level compared with the control group, we controlled the age and education effect for further analysis, but the statistical significance of differences in BV, GMR, WMR, BR, and CSFR between the patient and control groups remains unchanged.

In Pearson's correlation, sex, body mass index (BMI), Hct, MCV, AST, and GGT levels in the patient group, and age, sex, albumin, Hct, and GGT levels in the control group had significant correlations with at least 1 or more BV measurements (data not shown). We used stepwise linear regression analysis to determine the relationships of the above-mentioned significant variables with BV measurements in both the patient and control groups. The results are displayed in Table 3. Sex was significant in predicting BV and ICV in the patient and control groups, and also in WMR in the patient group. Age had significant effects on brain shrinkage, as measured by CSFR, BR, GMR, and WMR, among both patients and controls. In addition to age, BMI was significantly correlated with CSFR, BR, GMR, and WMR; GGT level correlated with BV, CSFR, BR, GMR, and WMR; Hct correlated with CSFR and BR in the patient group. No biological variables were correlated with BV measurements in the control group.

Table 4 shows significant variables correlated with brain shrinkage using stepwise regression analysis for the patient grouped by sex. Age significantly correlated with brain shrinkage in male patients but not in female patients. In male patients, serum GGT levels and Hct were significant variables correlated with brain shrinkage. In female patients, serum GGT and AST levels were significantly correlated with some BV measurements. No variables were correlated with ICV in either sex. Figure 1 shows the correlations between serum GGT levels and BR in male and female patients.

DISCUSSION

To the best of our knowledge, this is the first study to show a significant relationship between liver function as assessed using quantification of serum liver enzymes and BV as assessed using MRI measures in patients with alcohol dependence. In contrast, we did not find a significant relationship between liver function and BV among healthy control subjects. Significant relationships between levels of liver enzymes and BV measurements were present in both male and female patients with alcohol dependence. In addition, Hct was correlated with some BV measurements in male patients but not in female patients. The most striking finding is that levels of liver

Table 1. Clinical Characteristics and Laboratory Indexes in Alcoholic and Control Groups

	Alcoholic			Control			
	Total $N = 124$	Male $N = 79$	Female $N = 45$	Total $N = 111$	Male $N = 73$	Female $N = 38$	
	Mean ± SD			Mean ± SD			Group comparisons
Age (year)	40.0 ± 8.5	39.3 ± 8.4	41.4 ± 8.6	34.0 ± 9.8	32.7 ± 9.8	36.6 ± 9.4	AT > CT*, AM > CM*, AF = CF, CM = CF, AM = AF
Education	14.4 ± 2.4 ($n = 123$)	14.1 ± 2.3	15.0 ± 2.6 ($n = 44$)	16.5 ± 2.7 ($n = 106$)	16.6 ± 3.0 ($n = 70$)	16.3 ± 2.3 ($n = 36$)	AT < CT*, AM < CM*, AF = CF, CM = CF, AM = AF
BMI (kg/m^2)	25.0 ± 4.0	25.8 ± 3.7	23.6 ± 4.2	25.3 ± 4.4 ($n = 108$)	25.6 ± 4.7	24.8 ± 3.9	AT = CT, AM = CM, AF = CF, CM = CF, AM = AF
Drinking quantity in the last 180 days (kg of ethanol)	18.5 ± 9.2 ($n = 115$)	20.1 ± 9.9 ($n = 73$)	15.5 ± 7.1 ($n = 42$)	1.8 ± 1.5 ($n = 95$)	2.0 ± 1.6	1.4 ± 1.0	AT > CT*, AM > CM*, AF > CF*, CM = CF, AM > AF*
Lifetime drinking amount (kg of ethanol)	566.0 ± 484.0 ($n = 103$)	664.6 ± 549.1 ($n = 64$)	404.2 ± 292.7 ($n = 39$)	11.0 ± 14.7 ($n = 88$)	11.2 ± 14.8	10.9 ± 14.9	AT > CT*, AM > CM*, AF > CF*, CM = CF, AM > AF*
Age of onset	26.2 ± 9.1 ($n = 103$)	23.8 ± 8.2 ($n = 64$)	30.2 ± 9.1 ($n = 39$)	–			AM < AF*
Years of heavy drinking	11.6 ± 7.1 ($n = 103$)	13.3 ± 7.4 ($n = 64$)	8.8 ± 5.4 ($n = 39$)	–			AM > AF*
Smoking years	16.7 ± 11.7 ($n = 97$)	16.1 ± 10.6 ($n = 65$)	17.8 ± 13.7 ($n = 32$)	1.9 ± 4.3 ($n = 47$)	2.2 ± 4.6 ($n = 28$)	1.5 ± 3.9 ($n = 19$)	AT > CT*, AM > CM*, AF > CF*, CM = CF, AM > AF*
ALT[a] (U/l)	60.2 ± 59.3	66.1 ± 59.5	50.0 ± 58.1	24.1 ± 17.8	25.8 ± 18.8	20.7 ± 15.3	AT > CT*, AM > CM*, AF > CF*, CM > CF*, AM > AF*
AST[a] (U/l)	76.1 ± 83.2	79.1 ± 87.1	71.0 ± 76.6	22.0 ± 8.0	22.7 ± 8.0	20.5 ± 7.9	AT > CT*, AM > CM*, AF > CF*, CM = CF, AM = AF
GGT[a] (U/l)	219.2 ± 412.3	247.9 ± 493.1	169.0 ± 200.2	21.0 ± 9.8 ($n = 95$)	23.1 ± 10.1 ($n = 60$)	17.2 ± 7.9 ($n = 35$)	AT > CT*, AM > CM*, AF > CF*, CM > CF*, AM = AF
MCV (fl)	96.2 ± 5.7	95.3 ± 5.2	97.9 ± 6.2	88.5 ± 4.9	88.3 ± 4.0	88.8 ± 6.2	AT > CT*, AM > CM*, AF > CF*, CM = CF, AM < AF
Hct (%)	42.6 ± 4.4 ($n = 123$)	44.2 ± 4.0 ($n = 78$)	39.5 ± 3.6	42.4 ± 3.9	44.0 ± 2.9	39.2 ± 3.8	AT = CT, AM = CM, AF = CF, CM > CF*, AM > AF*
Albumin (g/dl)	4.4 ± 0.4 ($n = 122$)	4.4 ± 0.4 ($n = 77$)	4.4 ± 0.4	4.6 ± 0.3	4.6 ± 0.3	4.5 ± 0.3	AT = CT, AM < CM*, AF = CF, CM > CF*, AM = AF

AF, female alcoholic group; ALT, alanine aminotransferase; AM, male alcoholic group; AST, aspartate aminotransferase; AT, total alcoholic group; BMI, body mass index; CF, female control group; CM, male control group; CT, total control group; GGT, gamma-glutamyl transferase; Hct, hematocrit; and MCV, mean corpuscular volume of red blood cells.

[a]Comparisons between groups after these values were log-transformed.

*$p < 0.01$.

enzymes, either GGT or AST levels, are the only significant correlates, among the variables we measured in current study, of brain shrinkage in female patients.

The significantly positive correlation of serum GGT levels with CSFR and negative correlations with BR, GMR, and WMR indicated that serum GGT levels are associated with brain shrinkage. It suggests that GGT levels might directly or indirectly reflect the neurotoxic effect in both male and female patients. GGT has long been considered as a marker of alcohol-induced liver injury (Conigrave et al., 2003). Free radical production by ethanol has been implicated as a factor in its hepatotoxicity and most likely participates in the progression in alcohol-induced liver disease (Morimoto et al., 1993; Rashba-Step et al., 1993; Shaw et al., 1981). In animal studies, alcohol induced Kupffer cell to produce tumor necrosis factor-alpha (TNF-alpha) (Kono et al., 2000; Zhou et al., 2003). Elevated TNF-alpha has been reported in patients with alcohol dependence, especially those with alcohol-induced liver disease (Latvala et al., 2005), and is correlated with liver function impairment (Gonzalez-Quintela et al., 2008). Systemic TNF-alpha in patients with alcohol dependence can be transported from serum to the brain (Banks, 2005) and activate inflammatory responses (Qin et al., 2007). In vitro, TNF-alpha increased glutamate neurotoxicity via increased NF-kappaB DNA-binding activity (Zou and Crews, 2006). Although we did not measure TNF-alpha for our subjects, the increase in serum GGT may represent an indirect marker for increased oxidative stress. It suggests that levels of liver enzymes reflect oxidative stress in the liver, which is associated with TNF-alpha production and a possible neurotoxic effect on the brain. The correlation between TNF-alpha and brain shrinkage in patients with alcohol dependence needs to be explored in the future.

Another possible explanation for the relationship between serum GGT level and brain shrinkage is that increased serum GGT activity itself may be associated with oxidative stress (Whitfield, 2001). Although experimental studies have reported that cellular GGT has a central role in glutathione homeostasis by initiating the breakdown of extracellular glutathione, a critical antioxidant defense for the cell

Table 2. Brain Volumetric Indices Between Alcoholic and Control Groups

	Alcoholic			Control			
	Total $N = 124$	Male $N = 79$	Female $N = 45$	Total $N = 111$	Male $N = 73$	Female $N = 38$	
	Mean ± SD			Mean ± SD			Group comparisons
ICV	1,312.4 ± 121.4	1,350.2 ± 114.7	1,246.0 ± 104.0	1,345.3 ± 115.1	1,385.2 ± 99.7	1,268.5 ± 104.2	AT = CT, AM = CM, AF = CF, CM > CF*, AM > AF*
BV	1,031.2 ± 114.2	1,070.4 ± 103.6	962.3 ± 99.0	1,107.6 ± 101.4	1,142.1 ± 93.5	1,041.2 ± 81.7	AT < CT*, AM < CM*, AF < CF*, CM > CF*, AM > AF*
CSFR	0.28 ± 0.06	0.26 ± 0.05	0.30 ± 0.07	0.22 ± 0.04	0.21 ± 0.04	0.22 ± 0.04	AT > CT*, AM > CM*, AF > CF*, CM = CF, AM < AF*
BR	3.8 ± 0.8	3.9 ± 0.8	3.5 ± 0.8	4.8 ± 0.9	4.8 ± 0.9	4.8 ± 1.0	AT < CT*, AM < CM*, AF < CF*, CM = CF, AM > AF*
GMR	0.71 ± 0.07	0.72 ± 0.06	0.70 ± 0.08	0.78 ± 0.06	0.78 ± 0.06	0.79 ± 0.06	AT < CT*, AM < CM*, AF < CF*, CM = CF, AM = AF
WMR	0.59 ± 0.04	0.60 ± 0.03	0.57 ± 0.04	0.63 ± 0.03	0.63 ± 0.03	0.62 ± 0.03	AT < CT*, AM < CM*, AF < CF*, CM = CF, AM > AF*

AF, female alcoholic group; AM, male alcoholic group; AT, total alcoholic group; BR, brain ratio; BV, brain volume; CF, female control group; CM, male control group; CSFR, cerebrospinal fluid ratio; CT, total control group; GMR, gray matter ratio; ICV, intracranial volume; WMR, white matter ratio.

*$p < 0.01$.

Table 3. Significant Correlates with Various Brain Volumetric Indices in Alcoholic and Control Groups Using Stepwise Linear Regression Model[a]

	Alcoholic[b]					Control[c]	
	Age	Sex[d]	BMI	GGT	Hct	Age	Sex
ICV		−0.43					−0.49
BV		−0.48		−0.27			−0.47
CSFR	0.34		−0.22	0.43	−0.27	0.61	
BR	−0.41		0.25	−0.41	0.21	−0.57	
GMR	−0.43		0.30	−0.38		−0.62	
WMR	−0.25	−0.36	−0.20	−0.38		−0.40	

AST, aspartate aminotransferase; BMI, body mass index; BR, brain ratio; BV, brain volume; CSFR, cerebrospinal fluid ratio; GGT, gamma glutamyltransferase; GMR, gray matter ratio; Hct, hematocrit; ICV, intracranial volume; MCV, mean corpuscular volume of red blood cells; WMR, white matter ratio.

[a]Only shows beta values with $p < 0.01$.

[b]Brain morphology as dependent variable, and age, sex, BMI, Hct, MCV, AST, and GGT as independent variables for patient group.

[c]Brain morphology as dependent variable, and age, sex, albumin, Hct, and GGT as independent variables for control group.

[d]Male was coded as 1 and female was coded as 2.

Table 4. Significant Variables with Various Brain Volumetric Indices in Alcoholic Group by Gender Using Stepwise Linear Regression Model[a]

	Male			Female	
	Age	Hct	GGT	GGT	AST
ICV					
BV				−0.40	
CSFR	0.48	−0.28	0.33	0.52	
BR	−0.49	0.25	−0.32	−0.54	
GMR	−0.53	0.24	−0.29		−0.51
WMR	−0.32	0.28	−0.33	−0.55	

AST, aspartate aminotransferase; BMI, body mass index; BR, brain ratio; BV, brain volume; CSFR, cerebrospinal fluid ratio; GGT, gamma glutamyltransferase; GMR, gray matter ratio; Hct, hematocrit; ICV, intracranial volume; MCV, mean corpuscular volume of red blood cells; WMR, white matter ratio.

[a]Brain morphology as the dependent variable, and age, BMI, Hct, MCV, AST, and GGT as independent variables; only shows beta values with $p < 0.01$.

(Karp et al., 2001; Kugelman et al., 1994), there is evidence that under physiological conditions, GGT is directly involved in the generation of ROS, especially in the presence of iron or other transition metals (Drozdz et al., 1998; Enoiu et al., 2000; Zalit et al., 1996) and is associated with lipid peroxidation in hepatocytes (Paolicchi et al., 1997). Additionally, ALT, AST, and GGT levels have been shown to be positively correlated with serum MDA levels in patients with alcohol dependence (Huang et al., 2009). Because MDA is one of the reliable oxidative stress measures, the positive correlation suggests that serum GGT levels can at least partially reflect the severity of oxidative stress, which produces some toxic effects on the brain. Furthermore, it has been reported that serum GGT level is inversely correlated with serum antioxidants levels (Lim et al., 2004), and antioxidants can protect against binge-induced brain damage in vivo (Crews et al., 2006; Hamelink et al., 2005).

Among the correlation of levels of liver enzymes and MDA, correlation of ALT is the weakest (Huang et al., 2009). That might explain why ALT did not reach any significant correlation with brain morphology in patients with alcohol dependence in the current study. Another possible explanation is that GGT and AST exist in liver as well as in other organs, including brain, while ALT is relatively less affected by nonhepatic insults (Hanigan and Frierson, 1996; Pratt and Kaplan, 2000). It means the elevation of GGT and AST might not only come from liver injury, but also other organs, including brain, as well.

Nutritional effect might affect brain structural imaging. Brain lesions in patients with Wernicke's encephalopathy, which results from thiamin deficiency, include mammillary bodies, periaqueductal and periventricular area, and medial

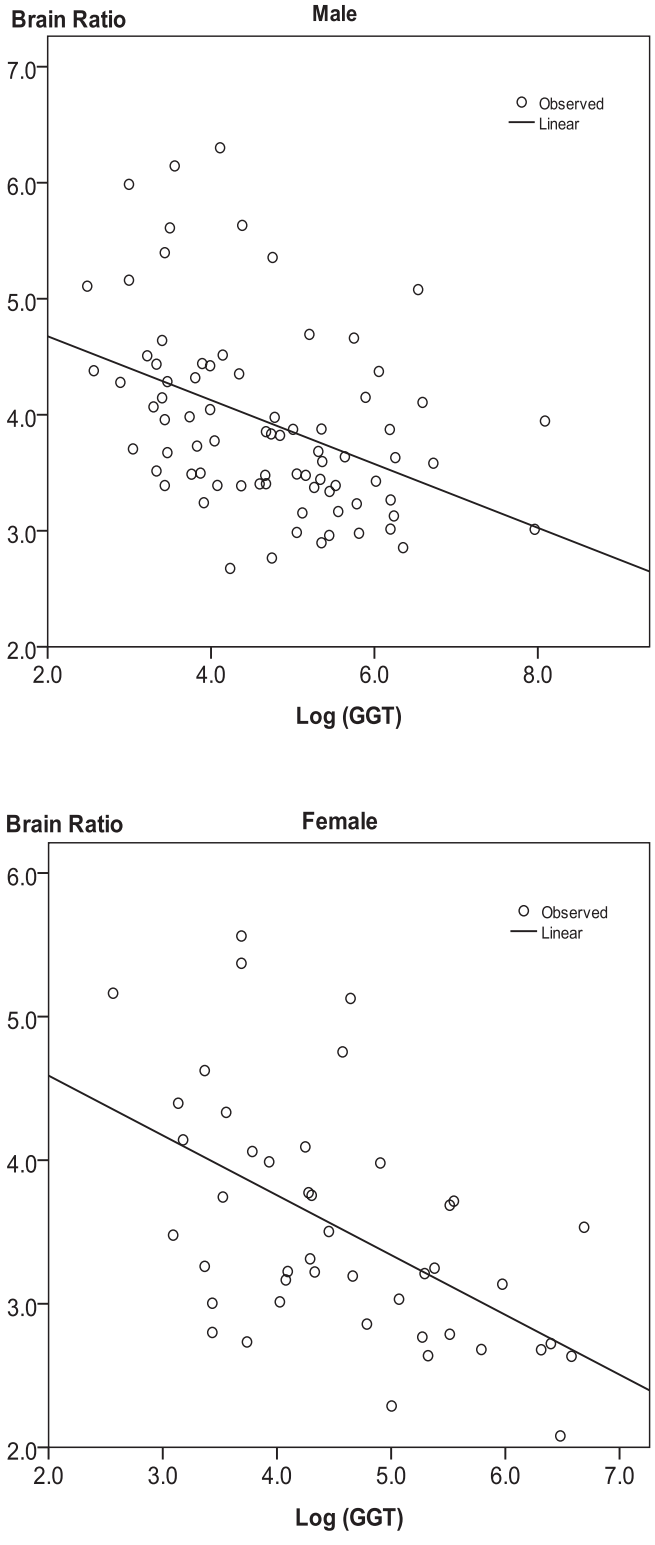

Fig. 1. Correlation between gamma-glutamyl transferase (GGT) and brain ratio in male and female patients with alcohol dependence.

thalami (Zuccoli et al., 2007). However, our subjects did not have Wernicke's encephalopathy, and their thiamin levels were within the normal range at admission. Therefore, the thiamine effect on BV measurements in our sample is minimal. In addition, other nutritional factors, such as Hct and albumin, have been shown to be associated with BV measurements in patients with alcohol dependence (Pfefferbaum et al., 2004). We included BMI, albumin, Hct, and MCV, which reflect patient's nutritional state, into our analyses. Among them, only Hct was significantly correlated with brain shrinkage in male patients, which is in accord with the results of previous studies (Pfefferbaum et al., 1988, 2004) but not in female patients. Our data also showed that liver function independent of nutritional status was significantly correlated with BV measurements in the patient group and that there were no any significant correlations between Hct and serum AST and GGT levels (data not shown). Therefore, it is unlikely that malnutrition mediates the relationship between liver function and brain shrinkage in the current study.

The age effect on brain volumetric measures in patients with alcohol dependence and controls has been documented in many studies (Hommer et al., 2001; Pfefferbaum et al., 1992, 2001), and there is some evidence of acceleration of aging among patients with alcohol dependence (Pfefferbaum et al., 2001). In our study, we found that the age effect was significant in both the patient and control groups with regard to CSFR, BR, GMR, and WMR. However, the age effect was absent in female patients when we stratified the analysis by sex. Among all variables we collected in this study, only liver functions, either serum GGT levels or AST levels, had significant correlations with BV measurements in female patients. Some have reported that female patients with alcohol dependence are more vulnerable to liver disease (Becker et al., 1996; Loft et al., 1987; Pares et al., 1986), cardiovascular disease (Fernandez-Sola et al., 1997; Urbano-Marquez et al., 1995), and alcohol-induced brain shrinkage (Hommer et al., 2001; Mann et al., 2005). In our study, we found that BV measures were more related to liver function measures than to aging in female patients.

There are some limitations to our study. First, there was a time lag, about 3 weeks, between serum tests and MRI examination. Liver function tests were performed within 24 hours of admission to the hospital so that we could obtain a measure of liver function associated with patients' usual pattern of drinking. MRI was performed after approximately 3 weeks of abstinence, by which time some recovery of the BV may have occurred (Pfefferbaum et al., 1995). The GGT also usually at least partially normalizes during the first several weeks of abstinence (Monteiro and Masur, 1986). Second, we used liver function indices, rather than directly measured ROS or oxidative damage markers such as MDA and 8-OHdG, to estimate severity of oxidative stress. Third, we did not have the information about past medication use, which might affect liver function tests. However, when we recruited these patients and control subjects, we excluded those who had medical or neurological disorders. The effect of medication should therefore be minimal if any.

In conclusion, our results showed that levels of liver enzymes, especially GGT, were highly correlated with brain shrinkage in patients with alcohol dependence but not in

control subjects. Because measurement of liver enzymes is reliable, easy, and inexpensive, compared with brain imaging study and oxidative stress markers, it can be used as an indirect indicator of the oxidative stress and possible brain insult. The underlying mechanisms of the relationship between liver function indices and brain shrinkage still need to be studied in the future.

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REFERENCES

Banks WA (2005) Blood-brain barrier transport of cytokines: a mechanism for neuropathology. Curr Pharm Des 11:973–984.

Becker U, Deis A, Sorensen TI, Gronbaek M, Borch-Johnsen K, Muller CF, Schnohr P, Jensen G (1996) Prediction of risk of liver disease by alcohol intake, sex, and age: a prospective population study. Hepatology 23:1025–1029.

Chen CH, Pan CH, Chen CC, Huang MC (2011) Increased oxidative DNA damage in patients with alcohol dependence and its correlation with alcohol withdrawal severity. Alcohol Clin Exp Res 35:338–344.

Conigrave KM, Davies P, Haber P, Whitfield JB (2003) Traditional markers of excessive alcohol use. Addiction 98(Suppl 2):31–43.

Courchesne E, Chisum HJ, Townsend J, Cowles A, Covington J, Egaas B, Harwood M, Hinds S, Press GA (2000) Normal brain development and aging: quantitative analysis at in vivo MR imaging in healthy volunteers. Radiology 216:672–682.

Crews FT, Nixon K (2009) Mechanisms of neurodegeneration and regeneration in alcoholism. Alcohol Alcohol 44:115–127.

Crews F, Nixon K, Kim D, Joseph J, Shukitt-Hale B, Qin L, Zou J (2006) BHT blocks NF-kappaB activation and ethanol-induced brain damage. Alcohol Clin Exp Res 30:1938–1949.

De Minicis S, Brenner DA (2008) Oxidative stress in alcoholic liver disease: role of NADPH oxidase complex. J Gastroenterol Hepatol 23(Suppl 1): S98–S103.

Drozdz R, Parmentier C, Hachad H, Leroy P, Siest G, Wellman M (1998) Gamma-glutamyltransferase dependent generation of reactive oxygen species from a glutathione/transferrin system. Free Radic Biol Med 25:786–792.

Enoiu M, Aberkane H, Salazar JF, Leroy P, Groffen J, Siest G, Wellman M (2000) Evidence for the pro-oxidant effect of gamma-glutamyltranspeptidase-related enzyme. Free Radic Biol Med 29:825–833.

Fataccioli V, Andraud E, Gentil M, French SW, Rouach H (1999) Effects of chronic ethanol administration on rat liver proteasome activities: relationship with oxidative stress. Hepatology 29:14–20.

Fein G, Di Sclafani V, Cardenas VA, Goldmann H, Tolou-Shams M, Meyerhoff DJ (2002) Cortical gray matter loss in treatment-naive alcohol dependent individuals. Alcohol Clin Exp Res 26:558–564.

Fernandez-Sola J, Estruch R, Nicolas JM, Pare JC, Sacanella E, Antunez E, Urbano-Marquez A (1997) Comparison of alcoholic cardiomyopathy in women versus men. Am J Cardiol 80:481–485.

Gonzalez-Quintela A, Campos J, Loidi L, Quinteiro C, Perez LF, Gude F (2008) Serum TNF-alpha levels in relation to alcohol consumption and common TNF gene polymorphisms. Alcohol 42:513–518.

Hamelink C, Hampson A, Wink DA, Eiden LE, Eskay RL (2005) Comparison of cannabidiol, antioxidants, and diuretics in reversing binge ethanol-induced neurotoxicity. J Pharmacol Exp Ther 314:780–788.

Hanigan MH, Frierson HF Jr (1996) Immunohistochemical detection of gamma-glutamyl transpeptidase in normal human tissue. J Histochem Cytochem 44:1101–1108.

Hommer DW, Momenan R, Kaiser E, Rawlings RR (2001) Evidence for a gender-related effect of alcoholism on brain volumes. Am J Psychiatry 158:198–204.

Huang MC, Chen CH, Peng FC, Tang SH, Chen CC (2009) Alterations in oxidative stress status during early alcohol withdrawal in alcoholic patients. J Formos Med Assoc 108:560–569.

Jernigan TL, Butters N, DiTraglia G, Schafer K, Smith T, Irwin M, Grant I, Schuckit M, Cermak LS (1991) Reduced cerebral grey matter observed in alcoholics using magnetic resonance imaging. Alcohol Clin Exp Res 15:418–427.

Junghanns K, Backhaus J, Veltrup C, Dageforde J, Bruckmann H, Wetterling T (2004) Mildly disturbed hepatic and pancreatic function during early abstention from alcohol is associated with brain atrophy and with disturbed psychometric performance. Alcohol Alcohol 39:113–118.

Karp DR, Shimooku K, Lipsky PE (2001) Expression of gamma-glutamyl transpeptidase protects ramos B cells from oxidation-induced cell death. J Biol Chem 276:3798–3804.

Kono H, Rusyn I, Yin M, Gabele E, Yamashina S, Dikalova A, Kadiiska MB, Connor HD, Mason RP, Segal BH, Bradford BU, Holland SM, Thurman RG (2000) NADPH oxidase-derived free radicals are key oxidants in alcohol-induced liver disease. J Clin Invest 106:867–872.

Kugelman A, Choy HA, Liu R, Shi MM, Gozal E, Forman HJ (1994) Gamma-glutamyl transpeptidase is increased by oxidative stress in rat alveolar L2 epithelial cells. Am J Respir Cell Mol Biol 11:586–592.

Latvala J, Hietala J, Koivisto H, Jarvi K, Anttila P, Niemela O (2005) Immune responses to ethanol metabolites and cytokine profiles differentiate alcoholics with or without liver disease. Am J Gastroenterol 100: 1303–1310.

Lim JS, Yang JH, Chun BY, Kam S, Jacobs DR Jr, Lee DH (2004) Is serum gamma-glutamyltransferase inversely associated with serum antioxidants as a marker of oxidative stress? Free Radic Biol Med 37:1018–1023.

Loft S, Olesen KL, Dossing M (1987) Increased susceptibility to liver disease in relation to alcohol consumption in women. Scand J Gastroenterol 22: 1251–1256.

Mann K, Ackermann K, Croissant B, Mundle G, Nakovics H, Diehl A (2005) Neuroimaging of gender differences in alcohol dependence: are women more vulnerable? Alcohol Clin Exp Res 29:896–901.

Mayne ST (2003) Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. J Nutr 133(Suppl 3):933S–940S.

McCrory SJ, Ford I (1991) Multivariate analysis of SPECT images with illustrations in Alzheimer's disease. Stat Med 10:1711–1718.

Momenan R, Hommer D, Rawlings R, Ruttimann U, Kerich M, Rio D (1997) Intensity-adaptive segmentation of single-echo T1-weighted magnetic resonance images. Hum Brain Mapp 5:194–205.

Monteiro MG, Masur J (1986) Monitoring alcoholism treatment: the appropriateness of choice between gamma GT or MCV evaluation after a short time of abstinence. Alcohol 3:223–226.

Morimoto M, Hagbjork AL, Nanji AA, Ingelman-Sundberg M, Lindros KO, Fu PC, Albano E, French SW (1993) Role of cytochrome P4502E1 in alcoholic liver disease pathogenesis. Alcohol 10:459–464.

Mutzell S (1992) Computed tomography of the brain, hepatotoxic drugs and high alcohol consumption in male alcoholic patients and a random sample from the general male population. Ups J Med Sci 97:183–194.

Paolicchi A, Tongiani R, Tonarelli P, Comporti M, Pompella A (1997) Gamma-glutamyl transpeptidase-dependent lipid peroxidation in isolated hepatocytes and HepG2 hepatoma cells. Free Radic Biol Med 22:853–860.

Pares A, Caballeria J, Bruguera M, Torres M, Rodes J (1986) Histological course of alcoholic hepatitis. Influence of abstinence, sex and extent of hepatic damage. J Hepatol 2:33–42.

Pfefferbaum A, Lim KO, Zipursky RB, Mathalon DH, Rosenbloom MJ, Lane B, Ha CN, Sullivan EV (1992) Brain gray and white matter volume loss accelerates with aging in chronic alcoholics: a quantitative MRI study. Alcohol Clin Exp Res 16:1078–1089.

Pfefferbaum A, Rosenbloom M, Crusan K, Jernigan TL (1988) Brain CT changes in alcoholics: effects of age and alcohol consumption. Alcohol Clin Exp Res 12:81–87.

Pfefferbaum A, Rosenbloom MJ, Deshmukh A, Sullivan EV (2001) Sex differences in the effects of alcohol on brain structure. Am J Psychiatry 158:188–197.

Pfefferbaum A, Rosenbloom MJ, Serventi KL, Sullivan EV (2004) Brain volumes, RBC status, and hepatic function in alcoholics after 1 and 4 weeks of sobriety: predictors of outcome. Am J Psychiatry 161:1190–1196.

Pfefferbaum A, Sullivan EV, Mathalon DH, Shear PK, Rosenbloom MJ, Lim KO (1995) Longitudinal changes in magnetic resonance imaging brain volumes in abstinent and relapsed alcoholics. Alcohol Clin Exp Res 19:1177–1191.

Pratt DS, Kaplan MM (2000) Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 342:1266–1271.

Qin L, Wu X, Block ML, Liu Y, Breese GR, Hong JS, Knapp DJ, Crews FT (2007) Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia 55:453–462.

Rashba-Step J, Turro NJ, Cederbaum AI (1993) Increased NADPH- and NADH-dependent production of superoxide and hydroxyl radical by microsomes after chronic ethanol treatment. Arch Biochem Biophys 300:401–408.

Shaw S, Jayatilleke E, Ross WA, Gordon ER, Leiber CS (1981) Ethanol-induced lipid peroxidation: potentiation by long-term alcohol feeding and attenuation by methionine. J Lab Clin Med 98:417–424.

Srivastava V, Buzas B, Momenan R, Oroszi G, Pulay AJ, Enoch MA, Hommer DW, Goldman D (2010) Association of SOD2, a mitochondrial antioxidant enzyme, with gray matter volume shrinkage in alcoholics. Neuropsychopharmacology 35:1120–1128.

Urbano-Marquez A, Estruch R, Fernandez-Sola J, Nicolas JM, Pare JC, Rubin E (1995) The greater risk of alcoholic cardiomyopathy and myopathy in women compared with men. JAMA 274:149–154.

Whitfield JB (2001) Gamma glutamyl transferase. Crit Rev Clin Lab Sci 38:263–355.

Zalit I, Glass GA, Stark AA (1996) The role of chelators in the catalysis of glutathione-gamma-glutamyl transpeptidase-dependent lipid peroxidation by transition metals. Biochem Mol Biol Int 40:1123–1133.

Zhou Z, Wang L, Song Z, Lambert JC, McClain CJ, Kang YJ (2003) A critical involvement of oxidative stress in acute alcohol-induced hepatic TNF-alpha production. Am J Pathol 163:1137–1146.

Zou J, Crews F (2006) CREB and NF-kappaB transcription factors regulate sensitivity to excitotoxic and oxidative stress induced neuronal cell death. Cell Mol Neurobiol 26:385–405.

Zuccoli G, Gallucci M, Capellades J, Regnicolo L, Tumiati B, Giadas TC, Bottari W, Mandrioli J, Bertolini M (2007) Wernicke encephalopathy: MR findings at clinical presentation in twenty-six alcoholic and nonalcoholic patients. AJNR Am J Neuroradiol 28:1328–1331.