

**Brain metabolism in minimal hepatic encephalopathy assessed by
3.0-Tesla magnetic resonance spectroscopy**

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Abstract:

To examine whether the brain exhibits metabolic disorder prior to overt hepatic encephalopathy in patients with liver cirrhosis (LC), the intracerebral glutamine and myo-inositol levels were determined using 3.0-Tesla (T) ^1H (proton)-magnetic resonance spectroscopy (MRS). We tested 21 LC patients, including seven patients with minimal hepatic encephalopathy (MHE). No significant differences were noted between the two patient groups in terms of the severity of liver cirrhosis, levels of blood ammonia or levels of blood or liver enzymes.

In the MHE group, the levels of brain glutamine were significantly higher than those in the non-MHE group, whereas the levels of brain myo-inositol were significantly lower. This demonstrated that MHE patients were already exhibiting metabolic disorder in the brain, similar to those observed during overt hepatic encephalopathy. Therefore, a quantitative analysis of this phenomenon using MRS may contribute to an early and objective diagnosis of MHE.

Keywords: glutamine, magnetic resonance spectroscopy, minimal hepatic encephalopathy, myo-inositol

Introduction:

Minimal hepatic encephalopathy (MHE), a pathological condition associated with slight neuro-psychological abnormalities detectable via sensitive and quantitative neuro-psychological tests (NPTs) ¹, is thought to include not only neuro-psychological cognitive dysfunction, but also the preliminary stages of overt encephalopathy. Thus, an early diagnosis of MHE could lead to the prevention of overt hepatic encephalopathy ². Although the mechanisms of the onset of hepatic encephalopathy and neuro-psychological dysfunction have been reported to be multifactorial ³ and many points remain unclear, ammonia is the leading candidate as a causative substance. The mechanism by which ammonia can lead to the onset of encephalopathy is assumed to be the accumulation of ammonia within the brain, which is metabolized and detoxified by glutamine synthetase in the astrocytes. This increase in glutamine causes edema of these cells ^{4, 5, 6, 7, 8}. An investigation using ¹H-MRS detected decreased levels of myo-inositol and increased levels of glutamine in the brain in patients with liver cirrhosis who had overt hepatic encephalopathy, and found that there was evidence of disordered brain metabolism ¹⁰. On the other hand, decreased levels of myo-inositol have been reported in studies using 1.5 T MRS in patients with MHE ¹¹. However, there have been few reports of the levels of glutamine, because studies using 1.5 T MRS cannot separate glutamine from the glutamine and glutamate complex (Glx). We have recently become able to separately measure the glutamine by using 3.0

T MRS.

Therefore, in the present study, we performed brain 3.0 T MRS using high magnetic field magnetic resonance imaging (MRI) on LC patients diagnosed with MHE in order to measure the metabolism (of glutamine and myo-inositol) within the brain, which could previously only be identified via clinical symptoms and tests. We thereby investigated whether it was possible to identify changes in the glutamine and myo-inositol levels in the brain even before overt hepatic encephalopathy was present.

Subjects:

The subjects consisted of 21 LC patients who were hospitalized or attending regular examinations at the Division of Gastroenterology and Hepatology, Department of Internal Medicine, Iwate Medical University [males, 15; females, 6; mean age, 60 years old (mean \pm SD; 60 \pm 12)]. LC was diagnosed based on diagnostic imaging studies, such as abdominal ultrasound and CT, and with histological or biochemical blood testing. The criteria for exclusion included a history of brain disease or mental disorders and overt encephalopathy ¹ of West Haven Criteria grade 1 or greater.

The causes of the disease were (in order from the most common) alcohol consumption (8 cases), viral infection (Hepatitis B, 1 case; Hepatitis C, 6 cases), autoimmune hepatitis (AIH)

(2 cases) and unknown (2 cases). The classification of the LC severity according to the Child Pugh Score (CPS) ¹ indicated that there were 14 grade A cases and seven grade B cases.

Methods:

Twenty-one subjects who exhibited no overt encephalopathy underwent NPTs. Four NPTs were conducted: the number connection tests A and B, the digit symbol test and the block design test. Patients who exhibited abnormalities for two or more tests were diagnosed with MHE ¹ (MHE group, 7 cases; non-MHE group, 14 cases). These patients underwent ¹H-MRS on the same day as the NPTs. The levels of glutamine (Gln) and myo-inositol (mIns) within the brain were measured in both groups and compared. When ¹H-MRS was performed, MRI (T1-SPGR, T2) was also performed, and it was confirmed that none of the subjects had an organic brain disease. A SIGNA 3.0 Tesla MR instrument (General Electric) was used to take high-resolution morphological images with the gradient field echo method, and single Voxel MRS (SV-MRS) region of interest (ROI) with a dimension of 20 × 20 × 20 mm was used in the occipital lobe gray matter and white matter. This location has the advantage of not being affected by brain atrophy or cerebral calcification. In addition, it contains both gray and white matter (Figure 1). SV-MRS was performed using point-resolved spectroscopy (PRESS), with a repeat time (TR) of 2000 msec, echo time (TE) of 80 msec and 128 as the number of excitations

(NEX). Spectra were analyzed using the LCMoel software program (Version 6.3-1J) ¹² to automatically quantify metabolites.

The levels of Gln and mIns were analyzed by fitting a linear combination of a basic set of metabolite model spectra to the data (LCMoel) (Figure 2). The Gln and mIns were expressed as ratios compared with creatine (Cre) and phosphocreatine (PCre) ¹³. We also measured the serum albumin level, prothrombin time (%), total bilirubin, blood ammonia and serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (γ -GTP). Based on these data, the severity of liver cirrhosis was evaluated with CPS and the model for end-stage liver disease (MELD) score. Comparisons for each item were then performed between the two groups (with and without MHE). The statistical analysis was conducted using the IBM SPSS software program version 22.0 (SPSS Inc., Chicago, IL, USA). The results are shown as the means \pm standard deviation (SD). Student's *t*-test was used for comparisons between the two groups. The Kruskal–Wallis test was used for multiple comparisons. A p-value <0.05 from the Kruskal–Wallis test was considered to be statistically significant. A regression analysis was performed to investigate the correlations among the data, and a p-value <0.05 was considered to be statistically significant.

This study was approved by the ethics review board of Iwate Medical University, and all subjects gave written informed consent prior to participation.

Results:

No organic diseases, such as cerebrovascular disease or brain tumors, were observed in any of the 21 subjects who underwent MRI. MRS indicated that the brain Gln levels were significantly increased ($p < 0.01$) in the MHE group (Gln/Cre + PCre: 2.05 ± 0.17) compared with the non-MHE group (Gln/Cre + PCre: 1.05 ± 0.09) (Figure 3).

In contrast, the brain mIns significantly decreased ($p < 0.01$) in the MHE group (mIns/Cre + PCre: 0.32 ± 0.04) compared with the non-MHE group (mIns/Cre + PCre: 0.57 ± 0.04) (Figure 4). We also observed a significant negative correlation ($r = 0.767$, $p < 0.01$) between the brain Gln and mIns levels (Figure 5). No significant differences were noted in the brain Gln levels with respect to the number of abnormalities in NPTs in the MHE group. The same trends were observed in the non-MHE group. The brain mIns levels were also not significantly different based on the number of abnormalities in NPTs (Table 1) (the Kruskal–Wallis test).

Blood tests revealed no significant differences between the MHE and non-MHE groups in the blood ammonia levels. However, a positive correlation ($r = 0.455$, $p < 0.05$) was noted between the blood ammonia and brain Gln levels (Figure 6), whereas a negative correlation ($r = 0.674$, $p < 0.05$) was noted between the blood ammonia and brain mIns levels

(Figure 7).

No significant differences were noted between the MHE and non-MHE groups for any of the other blood tests, the CPS or the MELD score (Table 2) (all $p > 0.05$ by Student's t -test).

In the regression analysis, the brain Gln levels had no relevance with regard to the various parameters examined, except for the brain mIns levels and blood ammonia level. In addition, the brain mIns levels were not correlated with any of the parameters except the brain Gln level and blood ammonia level.

Discussion:

We investigated the changes in the Gln and mIns levels measured using brain MRS in LC patients with and without MHE. The present results indicated that there is a marked increase in the brain Gln and a marked decrease in the mIns within the brain in patients with MHE compared with those without MHE. These data demonstrated that disturbed brain metabolism, similar to that observed in patients with overt hepatic encephalopathy, is also already present in patients with MHE. In addition, the results suggested that the quantification of these phenomena using MRS could be useful for an early and objective diagnosis of MHE.

A previous investigation using ^1H -MRS reported that the mIns decreases and the Gln increases in LC cases with hepatic encephalopathy ¹⁰. The mIns level is considered to be a

useful marker for diagnosing hepatic encephalopathy, because decreases in the mIns levels can reflect the presence of hepatic encephalopathy.

As shown above, the MHE cases in the present study also already exhibited decreased levels of mIns and increased levels of Gln, with significant differences compared with non-MHE patients. It has been shown that mIns, a phospholipid constituent found in brain cells, plays a role in maintaining the osmotic pressure within the brain, particularly within astrocytes¹⁴. The mechanisms responsible for the decrease in mIns and increase in Gln remain unknown, but Haussinger et al¹⁵. recently reported a hypothesized relationship between brain ammonia metabolism and the maintenance of the osmotic pressure within the brain. Brain ammonia metabolism is thought to occur in astrocytes, where ammonia is detoxified via the synthesis of Gln¹⁶. The increased Gln levels that arise following the metabolism of ammonia in the brain cause an increase in osmotic pressure within the brain. To regulate and maintain the osmotic pressure, mIns is secreted from the cells, which results in decreases in the mIns and increases in the Gln levels. This mechanism is presumed to underlie the changes in the levels of mIns and Gln⁶. However, the serum osmotic pressure in patients with liver cirrhosis is within the normal range and does not correlate with decreases in the mIns levels¹⁷. It has also been reported that toxic factors, such as ammonia, may be directly involved in the decrease in mIns levels¹⁸.

According to our investigation, we also observed that the blood ammonia level

exhibited a negative correlation with the mIns level and a positive correlation with the Gln level. In agreement with a previous report on patients with overt encephalopathy¹⁰, we confirmed that factors associated with ammonia were closely associated with the changes in mIns and Gln metabolism in MHE patients. However, since no significant difference was observed in the levels of blood ammonia between MHE and non-MHE patients, it was not an effective index for diagnosing MHE. In addition, although alcoholic cirrhosis was noted in eight of the present cases, significant differences between the groups with and without alcohol intake were not observed with regard to the MRS parameters.

In cases of LC with hepatic encephalopathy, disorders in Gln and mIns metabolism have been reported in various sites within the brain, such as the basal ganglia, temporal lobe and occipital lobe^{15, 19, 20}. Taylor-Robinson et al¹⁹ reported that the mean Choline/Cr was the lowest in the occipital cortex, and the mean Glx/Cr was the highest in the basal ganglia. In our study, we performed MRS on a predetermined region of interest in the occipital lobe because of the reason mentioned before, so it is unclear whether similar changes would be present at other sites. To confirm these changes in each region, it would be necessary to use multi-voxel MRS. It has been recently reported that disorders in brain Gln and mIns metabolism can be reversed by treatment. Haseler et al.²¹ compared the changes in the Gln and mIns levels before and after the administration of a synthetic disaccharide (lactulose 60 ml/day for seven days) with those of a

group not receiving the synthetic disaccharide. The results indicated that the group not receiving the synthetic disaccharide exhibited no improvement, while the group receiving the synthetic disaccharide exhibited a 15 % decrease in the Gln level and a 29 % increase in the mIns level. Another report indicated that these abnormalities improved after a liver transplant had been performed²², thus suggesting that MRS measurement may be useful for assessing the effects of treatment. It thus appears that in cases of LC, disorders in the metabolism of these substances in the brain already exist before the appearance of overt hepatic encephalopathy, and can improve following hepatic recovery. In addition, MRS may be useful for understanding the relevant pathophysiology of the condition, and ammonia may be related to the appearance of these abnormalities.

Moreover, the fact that our investigation indicated that there were significant differences between the MHE group and the non-MHE group in terms of the brain Gln and mIns levels in LC patients, even when no overt hepatic encephalopathy was observed, suggests that these changes affect the neuro-psychological functions.

There are several potential limitations associated with this study that should be kept in mind when interpreting the results. First, because of the small sample size, we were unable to adequately establish the applicability for diagnostic purposes. Second, no comparisons were made with healthy individuals. In a previous study, we compared the brain Gln levels between

healthy individuals and LC patients with no hepatic encephalopathy, and found that the Gln levels were significantly increased in the LC patients²³. However, we did not make comparisons among three groups (including a group of healthy individuals), and were therefore not able to compare the findings in the MHE cases with those of healthy individuals. We also did not perform follow-up observations or investigate the changes over time in the same patients after treatment. Therefore, whether changes in the brain Gln and mIns levels reflect changes in pathology cannot be thoroughly discussed. Finally, because the period when a diagnosis of LC was made could not be clearly classified for each case, the changes in the Gln and mIns levels were not sufficiently investigated in accordance with the LC stages.

Based on the above observations, further research needs to be conducted with a larger sample size, comparisons with healthy individuals and with follow-up observations of the same patients, and investigations should also be made of the changes associated with the MHE diagnosis over time. However, our present results suggest that the use of MRS is effective as a method to objectively and quantitatively evaluate patients to establish a diagnosis of MHE.

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Table 1. A comparison of the brain metabolites and the number of abnormalities in the NPTs

The number of abnormalities in NPTs		N	Gln/Cre+PCre	mins/Cre+PCre
Non-MHE	0	12	1.11 ± 0.31	0.55 ± 0.17
	1	2	0.65 ± 0.17	0.65 ± 0.20
MHE	2	4	1.92 ± 0.62	0.35 ± 0.14
	3	3	2.21 ± 0.34*	0.28 ± 0.04
	4	0	-	-

The number of abnormalities in NPTs *: 1vs.3 (p<0.05) by the Kruskal–Wallis test. Values are shown as the means ± SD

Table 2. The demographic, clinical and laboratory characteristics of the MHE and non-MHE groups

Study		MHE	Non MHE
Number		7	14
Age (years)		65 ± 9	58 ± 13
Male/female		5/2	10/4
Etiology			
Hepatitis C virus		1	5
Hepatitis B virus		0	1
Alcohol		3	5
Other		3	3
Child Pugh score A/B/C		3/4/0	10/4/0
Child Pugh score		6 ± 1	6 ± 1
MELD score		11 ± 2	10 ± 3
Biochemical parameters	[Reference range]		
Prothrombin activity (%)	[100.1 - 138.4]	53.2 ± 13.9	64.0 ± 17.2
Total bilirubin (mg/dL)	[0.2 - 1.2]	1.4 ± 1.0	1.2 ± 0.8
Albumin (g/dL)	[4.3 - 5.4]	3.6 ± 0.5	3.7 ± 0.6
Aspartate transaminase (IU/L)	[10.0 - 32.0]	45.7 ± 19.1	37.4 ± 17.3
Alanine transaminase (IU/L)	[7.0 - 27.0]	29.6 ± 8.7	29.5 ± 14.9
Alkaline phosphatase (IU/L)	[99.0 - 340.0]	492.3 ± 204.0	388.2 ± 99.5
Gamma-glutamyl transpeptidase (IU/L)	[5.0 - 55.0]	75.6 ± 31.2	101.6 ± 33.8
Plasma ammonia concentration(µg/dL)	[20.0 - 60.0]	119.0 ± 53.5	75.4 ± 40.8
MRS Parameters			
Glutamine/Creatin+Phosphocreatin*		2.05 ± 0.17	1.05 ± 0.09
Myo-inositol/Creatin+Phosphocreatin*		0.32 ± 0.04	0.57 ± 0.04

*p<0.01 by Student's t-test. Data are shown as the means ± SD

Figure legends

Figure 1. A cubic region of interest (ROI) for the SV-MRS analysis. The SV-MRS was performed at the ROI with a dimension of 20×20 mm, which was placed in the occipital lobe gray matter and white matter (boxed area).

Figure 2. A representative MRS spectrogram obtained using the LCModel software program. The peaks for both glutamine (Gln) and myo-inositol (mIns) were clearly identified, and the relative concentrations of those substances to creatine and phosphocreatine (Cr) were calculated.

Figures 3. A comparison of the brain glutamine (Gln) levels between the minimal hepatic encephalopathy (MHE) group and non-MHE group. Gln/Cre + PCre; glutamine creatine + phosphocreatine ratio

Figures 4. A comparison of the brain myo-inositol (mIns) levels between the minimal hepatic encephalopathy (MHE) group and non-MHE group. mIns/Cre + PCre; myo-inositol creatine + phosphocreatine ratio

Figures 5. The correlation between the brain glutamine (Gln) levels and brain myo-inositol (mIns) levels. Open circles and closed circles represent the MHE and non-MHE groups, respectively. Gln/Cre + PCre; glutamine creatine + phosphocreatine ratio, mIns/Cre + PCre; myo-inositol creatine + phosphocreatine ratio

Figure 6. The correlation between the brain glutamine (Gln) levels and blood ammonia levels. Open circles and closed circles represent the MHE and non-MHE groups, respectively. NH₃; blood ammonia, Gln/Cre + PCre; glutamine creatine + phosphocreatine ratio

Figure 7. The correlation between the brain myo-inositol (mIns) levels and blood ammonia levels. Open and closed circles represent the MHE and non-MHE groups, respectively. NH₃; blood ammonia, mIns/Cre + PCre; myo-inositol creatine + phosphocreatine ratio

Figure 1

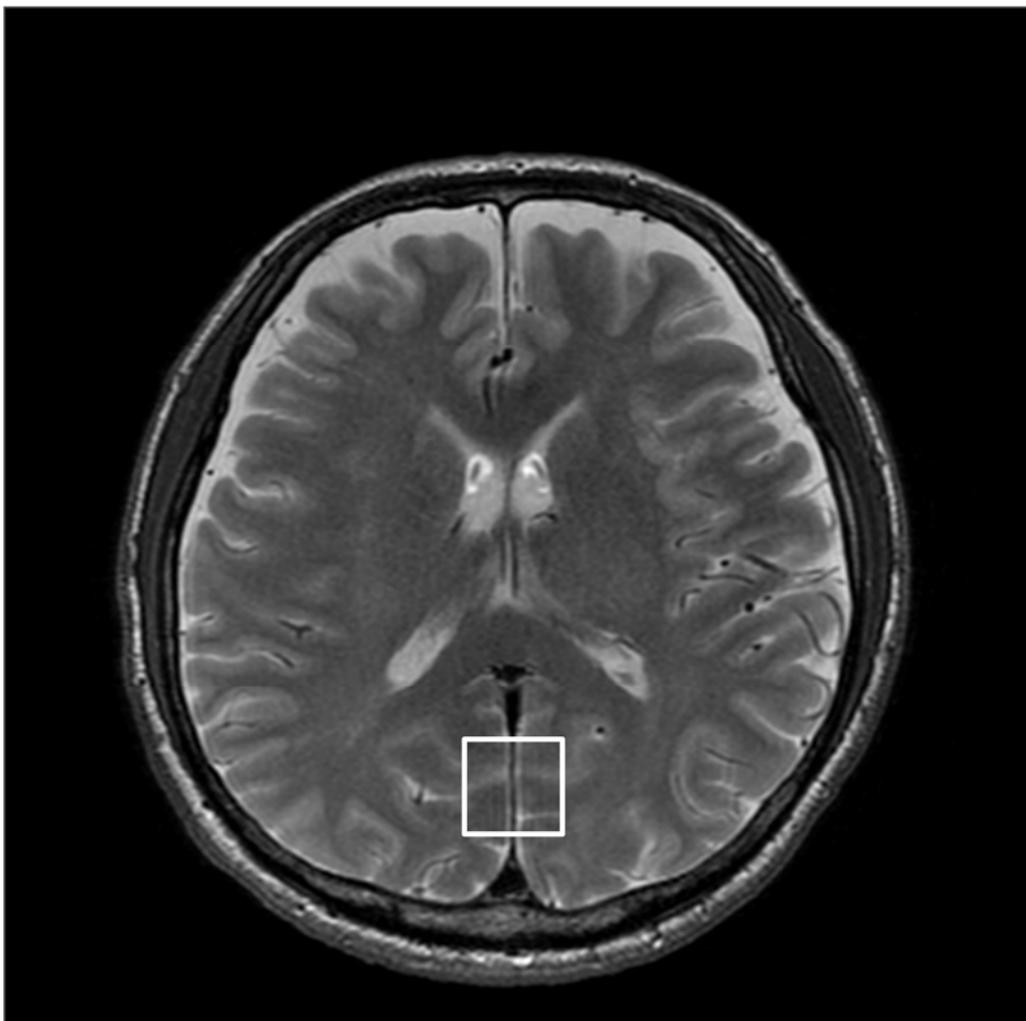


Figure 2

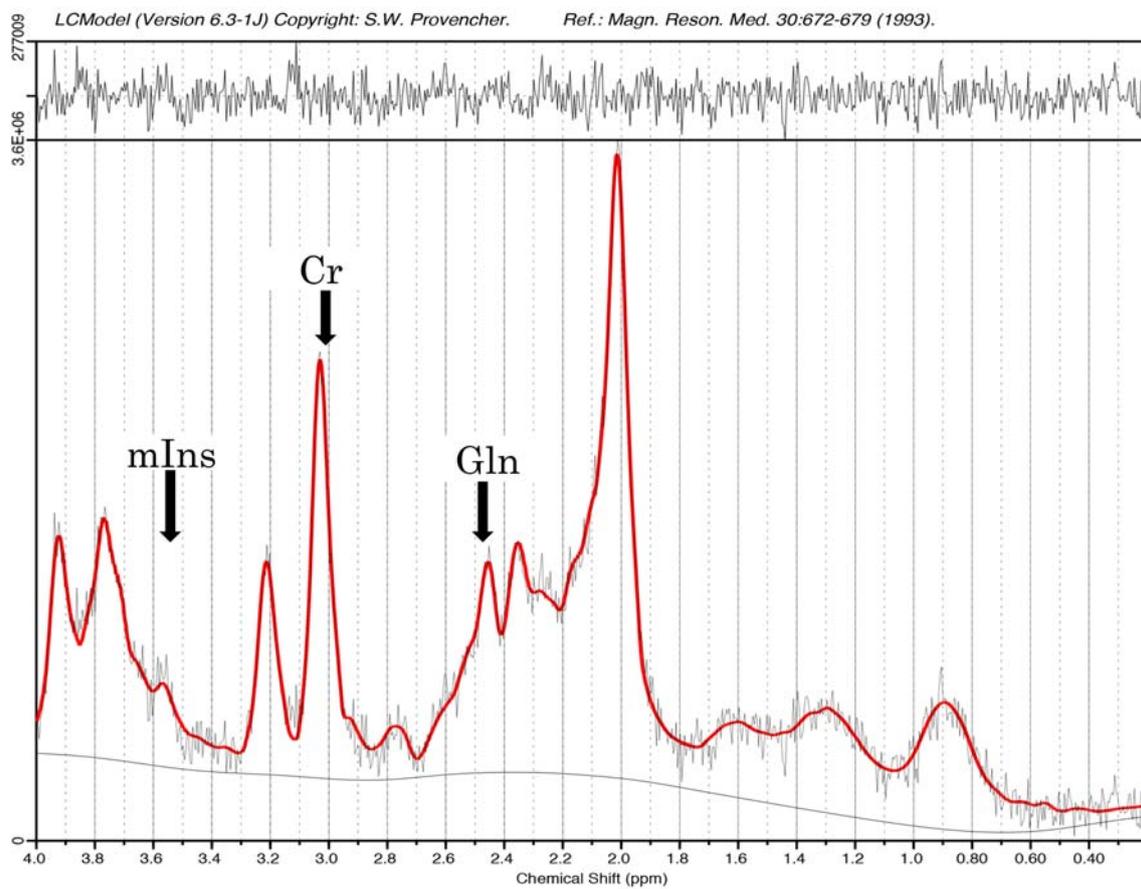


Figure 3

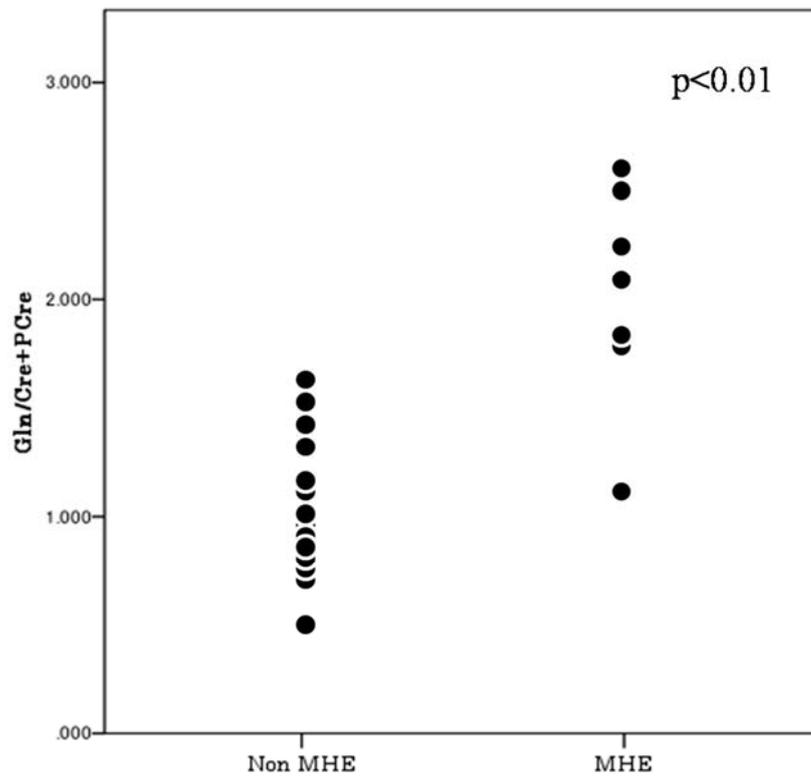


Figure 4

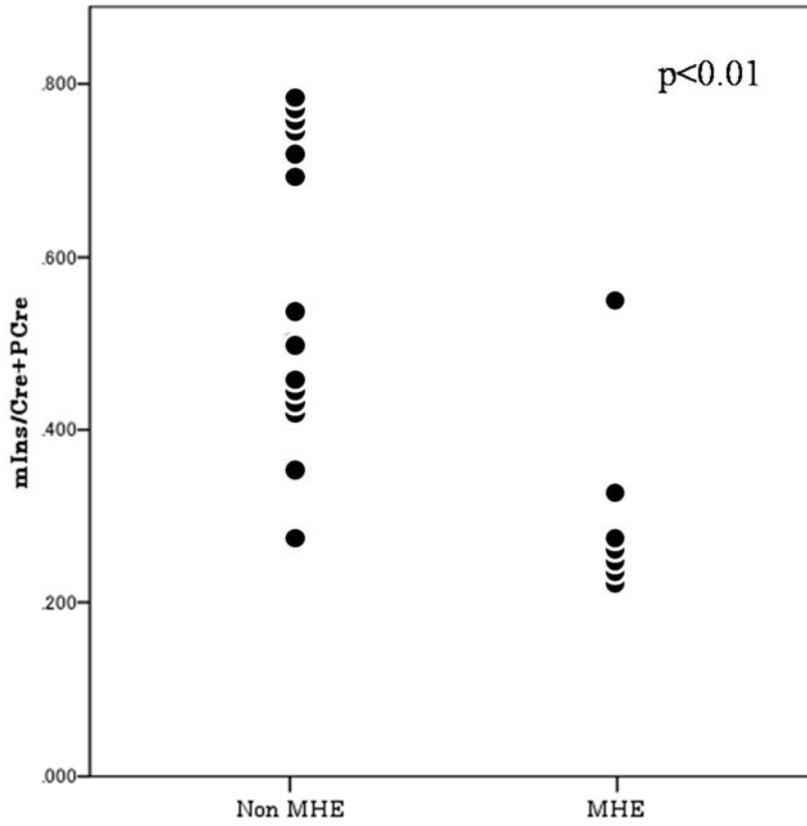


Figure 5

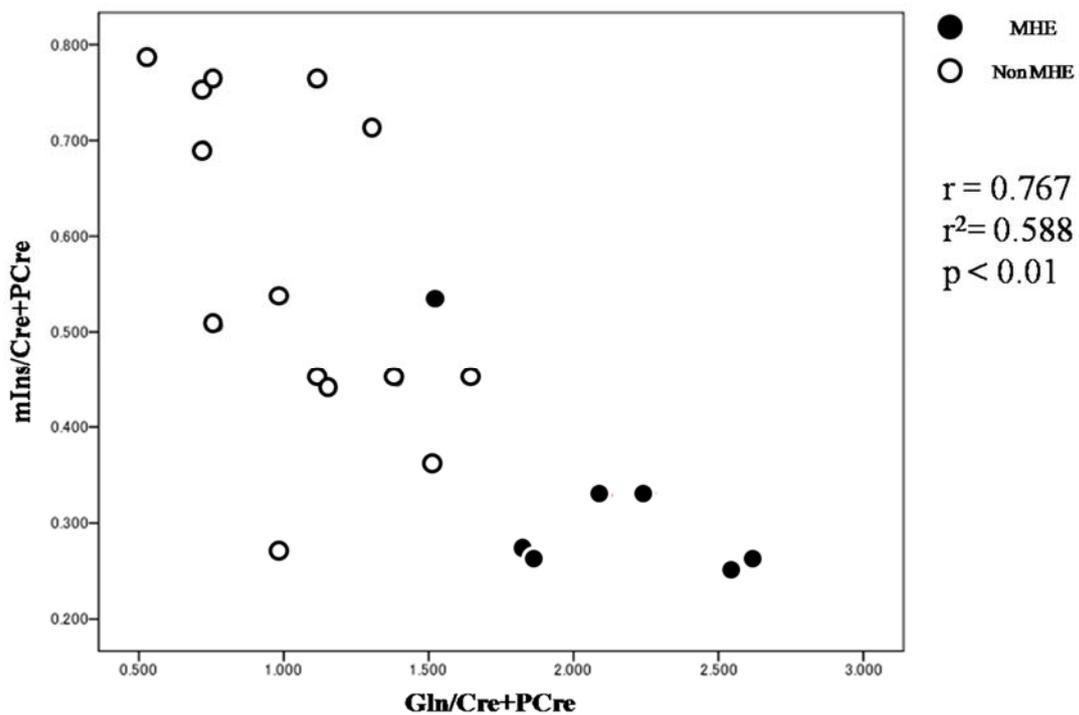


Figure 6

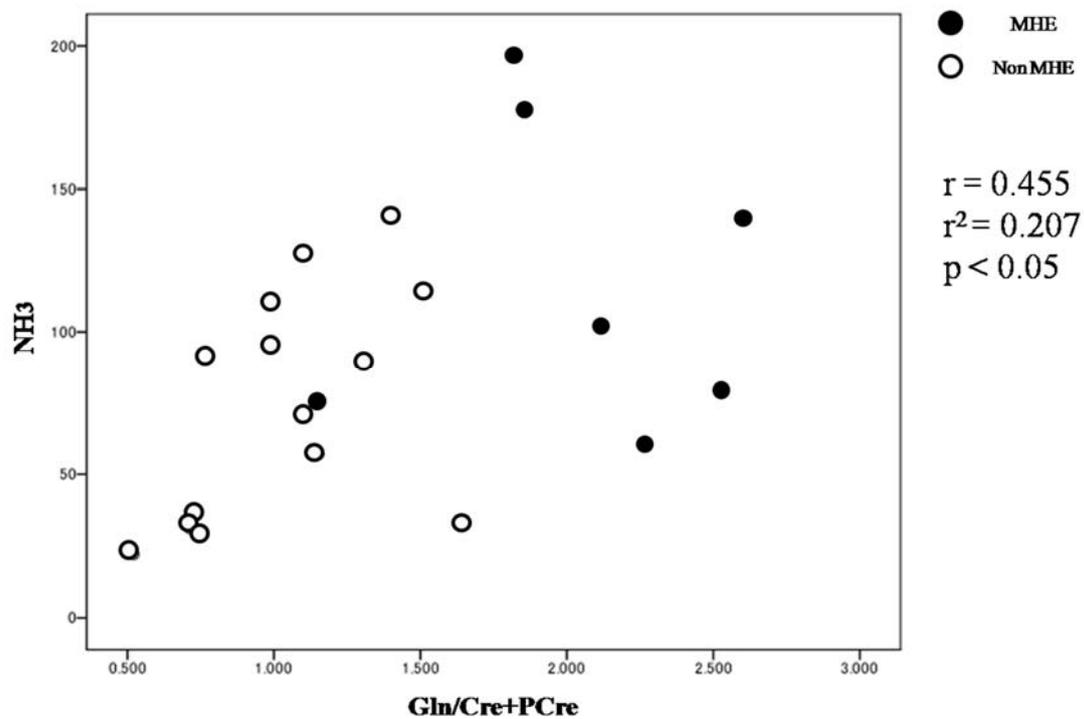


Figure 7

