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Does magnetic resonance spectroscopy identify patients with minimal hepatic encephalopathy?

Czy spektroskopia rezonansu magnetycznego identyfikuje pacjentów z minimalną encefalopatią wątrobową?

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Abstract

Background and purpose: The results of a few studies suggest that magnetic resonance spectroscopy of the brain could allow detection of minimal hepatic encephalopathy. The goal of this study was to assess the ability of magnetic resonance spectroscopy to differentiate between cirrhotic patients with and without minimal hepatic encephalopathy.
Material and methods: Localized magnetic resonance spectroscopy was performed in the basal ganglia, occipital gray matter and frontal white matter in 46 patients with liver cirrhosis without overt encephalopathy and in 45 controls. Neurological and neuropsychological examination was performed in each participant.
Results: The patients with liver cirrhosis had a decreased ratio of myoinositol to creatine in occipital gray matter and frontal white matter (mean: 0.17 ± 0.05 vs. 0.20 ± 0.04, $p = 0.01$ and 0.15 ± 0.05 vs. 0.19 ± 0.04, $p < 0.01$, respectively) and a decreased ratio of choline to creatine in occipital gray matter (mean: 0.32 ± 0.07 vs. 0.36 ± 0.08, $p = 0.03$). Minimal hepatic encephalopathy was diagnosed in 7 patients. Metabo-

Streszczenie

Wstęp i cel pracy: Wyniki pojedynczych badań sugerują, że spektroskopia rezonansu magnetycznego mózgu może być pomocna w wykrywaniu minimalnej encefalopatii wątrobowej. Celem tego badania była ocena przydatności spektroskopii rezonansu magnetycznego w odróżnianiu pacjentów z marskością wątroby z minimalną encefalopatią wątrobową od pacjentów bez tej encefalopatii.
Materiał i metody: Badanie spektroskopii rezonansu magnetycznego mózgu przeprowadzono u 46 pacjentów z marskością wątroby bez jawnej encefalopatii i u 45 osób z grupy kontrolnej. Rejestracji widm dokonano z trzech obszarów mózgu: zwojów podstawy, istoty szarej płata potylicznego i istoty białej płata czołowego. U wszystkich badanych osób przeprowadzono badanie neurologiczne i neuropsychologiczne.
Wyniki: U pacjentów z marskością wątroby stwierdzono obniżenie stosunku mioinozytolu do kreatyny w zakresie płata potylicznego i czołowego (średnie odpowiednio 0,17 ± 0,05 *vs* 0,20 ± 0,04, $p = 0,01$ oraz 0,15 ± 0,05 *vs* 0,19 ± 0,04, $p < 0,01$) oraz zmniejszenie stosunku choliny do kreatyny

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lite ratios did not differ significantly between patients with and without minimal hepatic encephalopathy. Metabolite ratios did not differ significantly between patients with Child-Pugh A and those with Child-Pugh B.

Conclusions: Magnetic resonance spectroscopy does not allow accurate diagnosis of minimal hepatic encephalopathy. A similar profile of metabolites in the brain is observed in cirrhotic patients without cognitive impairment.

Key words: magnetic resonance spectroscopy, minimal hepatic encephalopathy, liver cirrhosis, neuropsychological assessment.

w płacie potylicznym (średnia: 0,32 ± 0,07 *vs* 0,36 ± 0,08, $p = 0{,}03$) w porównaniu z grupą kontrolną. Minimalną encefalopatię wątrobową rozpoznano u 7 pacjentów. Stosunek metabolitów nie różnił się istotnie u pacjentów z minimalną encefalopatią wątrobową i bez niej. Nie stwierdzono także różnicy w stężeniu metabolitów u pacjentów z niewydolnością wątroby zakwalifikowanych do kategorii A w skali Child-Pugh w porównaniu z pacjentami zakwalifikowanymi do kategorii B.

Wnioski: Spektroskopia rezonansu magnetycznego nie pozwala na dokładne rozpoznanie minimalnej encefalopatii wątrobowej. Podobny profil metabolitów w mózgu obserwuje się u pacjentów z marskością wątroby bez zaburzeń poznawczych.

Słowa kluczowe: spektroskopia rezonansu magnetycznego, minimalna encefalopatia wątrobowa, marskość wątroby, ocena neuropsychologiczna.

Introduction

Proton magnetic resonance spectroscopy (MRS) allows *in vivo* measurement of different metabolites in the brain. MRS studies showed a decrease in the ratio of choline (Cho) to creatine (Cr) and myoinositol (Ins) to Cr and an increase in the ratio of glutamine/glutamate (Glx) to Cr in brains of patients with hepatic encephalopathy [1,2].

Minimal hepatic encephalopathy (MHE) is a subtle cognitive impairment commonly seen in patients with liver cirrhosis [3,4]. Identification of patients with MHE could be clinically important, because this group of patients has increased risk of overt encephalopathy and death [5,6]. Moreover, patients with MHE have reduced quality of life and deterioration in daily functioning including driving abilities [7]. The diagnosis of MHE is mainly based on performance of psychometric and/or neurophysiological tests. The results of neuropsychological and some neurophysiological tests could be, however, influenced by different factors including age, education, mood, degree of patient cooperation, etc. Taking into account these limitations, new methods allowing more objective identification of patients who are at risk of encephalopathy are needed.

Several studies have shown that patients with MHE have a decreased ratio of Ins/Cr and Cho/Cr and an increased ratio of Glx/Cr compared to controls [8-13]. Moreover, it was suggested that MRS allows accurate diagnosis of MHE [11,14]. In one study, MRS was able to differentiate patients with MHE from healthy controls with 100% accuracy when Ins depletion in the frontal and occipital lobe was used as a discriminating factor [11]. It should be noted that these studies compared patients with MHE only with healthy subjects. Therefore, it remains unclear whether MRS is able to differentiate patients with MHE from patients with liver cirrhosis without cognitive impairment.

The goal of our study was to assess the ability of MRS to differentiate between patients with liver cirrhosis with and without MHE.

Material and methods

The patients participating in this study were recruited from the patients with liver cirrhosis admitted from October 2008 to March 2011 to the outpatient clinic in the Department of Gastroenterology, Hepatology and Infectious Diseases, University Hospital in Krakow. The diagnosis of cirrhosis was based on clinical and biochemical data, results of ultrasound examination of the abdomen and, in selected cases, liver biopsy.

The exclusion criteria were: (1) overt hepatic encephalopathy or history of encephalopathy in the past; (2) history of psychiatric or neurological disorders; (3) history of recent use of drugs affecting psychometric performance (for example benzodiazepines, psychotropic drugs, etc.); (4) presence of structural changes on magnetic resonance imaging (MRI) which could interfere with results of spectroscopy or results of psychometric tests; (5) known central nervous system diseases, diabetes mellitus, severe cardiac and renal diseases; (6) active alcohol and drug abuse.

The inclusion criteria for control subjects were: 1) lack of history of neurological diseases; 2) normal result of neurological examination; 3) normal result of MRI of brain. The control group consisted of patients with functional symptoms from the gastrointestinal tract including patients with irritable bowel syndrome.

Neurological and neuropsychological examination was performed in all participants. The neuropsychological assessment included the Trail Making Test (TMT) part A and B, the Digit Symbol Test (DST) and the Block Design Test (BDT) from the Wechsler Adult Intelligence Scale Revised. These tests were chosen because they have a high specificity for diagnosing MHE and are recommended by the Working Party commissioned by the World Congress of Gastroenterology [15,16]. MHE was defined as the result of one or more tests below 2 standard deviations (SD) of the mean obtained in the control group.

^{1}HMRS was performed on a 1.5 T Magnetom Sonata (Siemens) scanner with single voxel PRESS technique (TR = 1500 ms, TE = 30 ms, 256 acquisitions with H_2O signal suppression). Three voxels of 8 cm^3 were positioned in the globus pallidus, predominantly white matter in the frontal lobe, and predominantly gray matter in the occipital cortex. Metabolite concentrations were calculated manually, using integral Siemens software. Peaks from Ins (position 3.54 ppm), Cho (position 3.24 ppm) and N-acetyl-aspartate (NAA) (position 2.02 ppm) and Glx (integrated area under peaks 2.15, 2.23, 2.30 ppm) were normalized with respect to Cr (position 3.04 and 3.93). According to the previous studies, it was assumed that creatine levels are not changed in the course of hepatic encephalopathy.

MR was performed using the same machine (T1-weighted images: TR 4000 ms/TE 122 ms; T2-weighted images: TR 390 ms/TE 7.7 ms; FLAIR: TR 1000 ms/TE 121 ms). Brightness of basal ganglia was determined visually on T1-weighted images and classified as normal or increased signal intensity of the globus pallidus.

The study protocol was approved by the Bioethics Committee of the Jagiellonian University and informed consent was obtained from each participant.

The χ^2 test was used to compare proportions, Mann-Whitney *U*-test to compare continuous variables between two groups and Kruskal-Wallis test to compare continuous variables between three groups. For correlation analysis, Spearman's rank correlation test was used. *P*-values of less than 0.05 were considered to indicate statistical significance. The calculations were performed using the statistical package STATISTICA for Windows, v.9.0 (StatSoft, Inc).

Results

Forty-six patients with liver cirrhosis without overt encephalopathy and 45 control subjects were enrolled. The causes of liver cirrhosis were: hepatitis B (11 patients) and hepatitis C (10 patients) infections, alcohol abuse (6 patients), autoimmune hepatitis (16 patients), non-alcoholic steatohepatitis (1 patient), primary sclerosing cholangitis (1 patient) and haemochromatosis (1 patient). According to the Child-Pugh scale of hepatic insufficiency, 26 patients were classified as having Child-Pugh A disease, 19 patients were classified as Child-Pugh B, and 1 patient as Child-Pugh C.

Demographic and laboratory characteristics as well as results of neuropsychological tests are shown in Table 1. There was no significant difference in age, sex or education between studied groups. Compared to controls, the patients with liver cirrhosis had higher levels of aminotransferases, alkaline phosphatase and gamma-glutamyl transpeptidase in serum. Ammonia level in serum did not differ significantly between groups.

There was no significant difference in results of neuropsychological tests between patients and control subjects.

The spectra from the globus pallidus and occipital cortex were obtained in all participants; spectra from the frontal lobe were obtained in 39 patients and 38 controls. The metabolite ratios are shown in Table 2 and Table 3. First, the group of all patients (those without abnormalities on neuropsychological examination and those with MHE) was compared with the control group. The patients with liver cirrhosis had a decreased ratio of Ins/Cr in occipital gray matter and frontal white matter (mean: 0.17 ± 0.05 vs. 0.20 ± 0.04, $p = 0.01$ and 0.15 ± 0.05 vs. 0.19 ± 0.04, $p < 0.01$, respectively), decreased ratio of Cho/Cr in occipital gray matter (mean: 0.32 ± 0.07 vs. 0.36 ± 0.08, $p = 0.03$) and increased ratio of NAA/Cr in the globus pallidus (mean: 1.95 ± 0.32 vs. 1.83 ± 0.34, $p = 0.04$). When three groups (patients without abnormalities on neuropsychological examination, patients with MHE and control subjects) were compared, ANOVA analysis revealed that patients with MHE had a significantly decreased ratio of Cho/Cr in the occipital lobe compared with the control group and patients with liver cirrhosis without MHE had a decreased ratio of Ins/Cr in the frontal

Table 1. Demographic, laboratory and neuropsychological data of patients with liver cirrhosis and control group

	Patients (N = 46)	Controls (N = 45)	P-value
Age [years], mean (SD)	39.8 (10.4)	40.3 (13.2)	0.90
Female, *n* (%)	23 (50.0)	23 (51.1)	0.76
Education [years], mean (SD)	13.8 (2.7)	13.9 (2.7)	0.73
Biochemical parameters			
Bilirubin [mmol/L], mean (SD) [normal value: 0.0-17.1]	18.1 (15.2)	13.1 (7.1)	0.09
Aspartate transaminase [U/L], mean (SD) [normal value: 10-40]	59.1 (48.6)	25.7 (13.0)	< 0.01
Alanine transaminase [U/L], mean (SD) [normal value: 10-41]	96.7 (120.8)	32.4 (22.4)	< 0.01
Gamma-glutamyl transpeptidase [U/L], mean (SD) [normal value: 5.0-61.0]	186.4 (255.2)	34.1 (28.6)	< 0.01
Cholinesterase [U/L], mean (SD) [normal value: 5320-12920]	8363 (2821)	9095 (2208)	0.24
Alkaline phosphatase [U/L], mean (SD) [normal value: 91-258]	231.3 (150.1)	147.5 (85.1)	< 0.01
Albumin [g/L], mean (SD) [normal value: 35.0-50.0]	45.7 (3.9)	47.2 (4.4)	0.23
Ammonia [μg/dL], mean (SD) [normal value: 9-33]	37.6 (19.7)	22.2 (7.3)	0.66
Prothrombin [INR], mean (SD) [normal value: 0.9-1.2]	1.1 (0.2)	1.0 (0.1)	0.04
Urea [mmol/L], mean (SD) [normal value: 1.7-8.3]	5.1 (1.3)	5.0 (1.4)	0.66
Platelets [$1 \times 10^3/\mu$L], mean (SD) [normal value: 125-340]	198 (80)	220 (56)	0.30
Neuropsychological tests			
TMT-A [s], mean (SD)	29.1 (14.4)	27.9 (8.6)	0.47
TMT-B [s], mean (SD)	70.9 (40.1)	68.5 (36.2)	0.77
DST, mean (SD)	51.1 (12.1)	53.9 (12.0)	0.41
BDT, mean (SD)	31.5 (8.4)	32.9 (8.3)	0.52

SD – standard deviation; TMT – Trail Making Test; DST – Digit Symbol Test; BDT – Block Design Test

and occipital lobe compared with control subjects. Metabolite ratios did not differ significantly between patients with and without MHE, although there was a statistical trend ($p = 0.08$) toward a lower ratio of Cho/Cr in the occipital lobe in patients with MHE.

Metabolite ratios did not differ significantly in patients with Child-Pugh A and those with Child-Pugh B (Table 3).

In patients with liver cirrhosis there was no significant linear correlation between serum ammonia level and metabolite ratios. INR correlated significantly ($p < 0.05$) with Ins/Cr ratio in the globus pallidus (R = –0.33), occipital cortex (R = –0.38) and frontal lobe (R = –0.44) as well as Cho/Cr ratio in the globus pallidus (R = –0.31).

Bright basal ganglia were found in 4 patients. These patients did not differ significantly in neuropsychological tests from those with normal signal intensity of the globus pallidus. Also biochemical parameters did not differ significantly between these groups, with the exception of cholinesterase activity, which was lower in patients with bright basal ganglia (mean: 8776 ± 2859 vs. 5479 ± 2085 U/L, $p = 0.02$). Patients with bright basal ganglia had a decreased ratio of Cho/Cr and Ins/Cr in the globus pallidus (mean: 0.56 ± 0.15 vs. 0.78 ± 0.15, $p = 0.01$ and 0.16 ± 0.08 vs. 0.26 ± 0.08, $p = 0.03$) and a decreased ratio of Ins/Cr in the occipital cortex (0.11 ± 0.05 vs. 0.17 ± 0.05, $p = 0.04$). There was also a statistical trend towards a lower ratio of Ins/Cr in the frontal lobe (0.09 ± 0.07 vs. 0.16 ± 0.05, $p = 0.08$).

Discussion

In this study, metabolite ratios did not differ significantly between patients with liver cirrhosis with and without MHE. Previous studies measuring the metabolites in brains of patients with MHE brought equivo-

Table 2. Metabolite ratios (mean [SD]) in patients with liver cirrhosis without encephalopathy, patients with minimal encephalopathy and control subjects

	Patients with liver cirrhosis without encephalopathy (group 1)	Patients with minimal encephalopathy (group 2)	Control group (group 3)	*P*-value	Post-hoc comparisons
Globus pallidus	$N = 39$	$N = 7$	$N = 45$		
NAA/Cr	1.94 (0.31)	1.99 (0.38)	1.83 (0.34)	0.12	
Cho/Cr	0.75 (0.15)	0.78 (0.23)	0.75 (0.14)	0.51	
Ins/Cr	0.24 (0.07)	0.28 (0.13)	0.26 (0.07)	0.09	
Glx/Cr	0.85 (0.25)	0.99 (0.18)	0.93 (0.31)	0.20	
Occipital lobe	$N = 39$	$N = 7$	$N = 45$		
NAA/Cr	1.06 (0.20)	1.11 (0.25)	1.12 (0.22)	0.34	
Cho/Cr	0.33 (0.07)	0.27 (0.06)	0.36 (0.08)	0.02	group 3 > group 2
Ins/Cr	0.17 (0.05)	0.15 (0.07)	0.20 (0.04)	0.04	group 3 > group 1
Glx/Cr	1.01 (0.23)	0.86 (0.40)	1.05 (0.29)	0.48	
Frontal lobe	$N = 34$	$N = 5$	$N = 38$		
NAA/Cr	1.17 (0.35)	0.91 (0.24)	1.04 (0.25)	0.11	
Cho/Cr	0.45 (0.12)	0.42 (0.09)	0.47 (0.09)	0.53	
Ins/Cr	0.15 (0.05)	0.16 (0.09)	0.19 (0.04)	< 0.01	group 3 > group 1
Glx/Cr	1.07 (0.35)	1.12 (0.04)	1.23 (0.35)	0.24	

NAA – N-acetyl-aspartate; Cho – choline; Cr – creatine; Ins – myo-inositol, Glx – glutamine/glutamate

Table 3. Metabolite ratios (mean [SD]) according to the Child-Pugh scale

	Child-Pugh A	Child-Pugh B	*P*-value
Globus pallidus	$N = 26$	$N = 19$	
NAA/Cr	1.91 (0.30)	2.00 (0.34)	0.43
Cho/Cr	0.79 (0.15)	0.72 (0.18)	0.40
Ins/Cr	0.25 (0.07)	0.24 (0.10)	0.60
Glx/Cr	0.87 (0.25)	0.87 (0.24)	0.91
Occipital lobe	$N = 26$	$N = 19$	
NAA/Cr	1.04 (0.21)	1.11 (0.20)	0.18
Cho/Cr	0.32 (0.07)	0.32 (0.07)	0.74
Ins/Cr	0.17 (0.04)	0.15 (0.06)	0.77
Glx/Cr	1.03 (0.25)	0.96 (0.25)	0.41
Frontal lobe	$N = 22$	$N = 16$	
NAA/Cr	1.14 (0.22)	1.14 (0.48)	0.32
Cho/Cr	0.48 (0.11)	0.42 (0.12)	0.13
Ins/Cr	0.16 (0.05)	0.14 (0.05)	0.50
Glx/Cr	1.07 (0.39)	1.07 (0.25)	0.99

NAA – N-acetyl-aspartate; Cho – choline; Cr – creatine; Ins – myo-inositol, Glx – glutamine/glutamate

cal results. Geissler *et al.* [8] found a decreased ratio of Ins/Cr in gray and white matter and an increased ratio of Glx/Cr in gray matter in 21 patients with MHE compared to healthy volunteers. The same changes in brain metabolites accompanied by a decreased ratio of Cho/Cr in white matter were also observed in patients with liver cirrhosis with normal results of psychometric tests. Laubenberger *et al.* [9] observed a decreased ratio of Ins/Cr in gray and white matter and an increased ratio of glutamine/Cr in gray matter in 4 patients with MHE compared to controls. In that study, no significant difference was found between Ins and glutamine concentrations of asymptomatic patients and those with MHE. Singhal *et al.* [11] used two-dimensional MRS to demonstrate a decrease in Ins and Cho and an increase in Glx in occipital and frontal lobes of patients with MHE compared with healthy subjects. The same profile of metabolites was also detected in the anterior cingulated region [10]. Rovira [12] compared metabolite levels in 7 patients with MHE and 16 patients with liver cirrhosis without cognitive impairment. They found that ratios of Ins/Cr +phospho-Cr in white matter were significantly more decreased and ratios of Glx/Cr +phospho-Cr were increased in cirrhotic patients with MHE

compared to those without MHE. In another study, Weissenborn [13] compared MRS data obtained from 9 patients with liver cirrhosis and without cognitive disturbances, 6 patients with MHE and 6 patients with hepatic encephalopathy grade I. ANOVA analysis showed a difference between these 3 groups in ratios of Ins/Cr and Cho/Cr with the lowest value of these metabolites in patients with MHE. There was also a statistical trend ($p = 0.07$) for an increased ratio of Glx/Cr, with the highest value in patients with hepatic encephalopathy. The changes in metabolite concentrations were seen only in white matter. Although direct comparison of published studies is not possible due to differences in study design (definition of MHE, MRS acquisition and analysis protocols, etc.), some general conclusions can be drawn. The cited studies demonstrated that patients with MHE have decreased ratios of Ins/Cr and Cho/Cr and an increased ratio of Glx/Cr compared to healthy subjects. However, a similar profile of metabolites could be seen in patients with liver cirrhosis without cognitive disturbances. Our results and the results of some previous reports [8,9] suggest that there is no significant difference in metabolite ratios between patients with MHE and patients with liver cirrhosis without cognitive impairment. In contrast, some studies have shown quantitative differences between patients with and without MHE [12,13]. Even in these studies, there were significant overlaps in metabolite concentrations between patients with and without MHE. Therefore, it seems that MRS is not helpful in differentiation between these two groups of patients without overt encephalopathy.

MRS is a rapidly growing area of technological advances. In our study we measured only a limited number of metabolites. We cannot rule out the possibility that changes in other metabolites, not studied by us, could be important for differentiating patients with and without MHE.

In this study patients with liver cirrhosis had a decreased ratio of Ins/Cr in occipital gray matter and frontal white matter and a decreased ratio of Cho/Cr in occipital gray matter. These results suggest that the depletion of Ins is an early phenomenon that precedes the increase in Glx.

Some previous studies have suggested that the changes in metabolite levels in the brain reflect the chronic metabolic derangement associated with the hepatic functional reserve [13,17]. The Ins level was inversely correlated with the Child-Pugh score. Also the Co level was lower and the Glx level was higher in patients with Child-Pugh B/C than in patients with Child-Pugh A. However, these data were obtained from a group of patients with liver cirrhosis that also included persons with overt hepatic encephalopathy. We did not find differences in metabolite levels between patients with Child-Pugh A and B. This may suggest that in patients without overt encephalopathy who have a relatively low serum ammonia level, factors other than the degree of liver dysfunction determine metabolite ratios in the brain.

The presence of signal hyperintense basal ganglia in T1-weighted images in patients with liver cirrhosis is related to deposition of manganese [18]. We found that patients with bright basal ganglia had lower ratios of Ins/Cr in gray and white matter and a lower ratio of Cho/Cr in basal ganglia. This may suggest that pallidal hyperintensities are visible when Ins depletion reaches a certain degree.

Conclusions

Our results suggest that MRS does not allow accurate diagnosis of MHE, because a similar profile of metabolites in the brain is observed in patients with liver cirrhosis without cognitive impairment. To draw firm conclusions regarding the utility of MRS in patients with MHE, a larger group of patients is needed.

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Disclosure

Authors report no conflict of interest.

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