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Brain magnetic resonance spectroscopy (MRS) as a diagnostic tool for detecting early neurological changes in children with Wilson's disease

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ABSTRACT

Purpose: Although brain magnetic resonance spectroscopy (MRS) imaging findings in adult Wilson disease (WD) have been explained in extensive details, a paucity of information currently exists regarding brain MRS imaging findings in pediatric WD. The purpose of this study was to clarify the role of brain MRS in detecting early metabolite abnormalities in children with WD.

Patient and methods: A case-controlled prospective study included 26 children with WD and 26 healthy controls. All children were subjected to examination on a 1.5 T MRI scanner. The spectra of N-acetyl aspartate (NAA), choline (Cho), and creatine (Cr), as well as the metabolite ratios of NAA/Cho, NAA/Cr, and Cho/Cr, were measured and compared between two groups.

Results: Eight patients revealed increased signal intensity in the basal ganglia at T1-weighted images. When compared with healthy controls, WD patients showed a significant decrease ($p < 0.05$) in NAA (63.8 ± 9.6 vs 97.6 ± 3.8), Cho (46.7 ± 8.9 vs 87.3 ± 4.7), Cr (44 ± 10.1 vs 81.9 ± 4.05), NAA/Cho (1.92 ± 1.2 vs 3.34 ± 0.55), NAA/Cr (1.29 ± 0.7 vs 2.46 ± 0.34), and Cho/Cr (0.78 ± 0.4 vs 2 ± 0.13). Patients complicated with liver cell failure showed a significant decrease in all previous parameters ($p < 0.05$) than patients without complications. Patients with mixed neurological and hepatic diseases showed a severe reduction in NAA, NAA/Cr, and NAA/Cho compared with patients with hepatic disease only.

Conclusion: MRS in pediatric WD detects early neurological changes even with normal MRI.

1. Introduction

Wilson's disease (WD) is a hereditary autosomal recessive disease of copper metabolism characterized by the accumulation of copper in the liver, brain, kidneys and other body organs. WD is rare with world incidence of approximately 1:50/100.000 live births [1]. The main clinical presentations of WD are hepatic and neuropsychiatric features. The hepatic presentations range from biochemical abnormalities only to acute hepatic failure. The neurological manifestations include tremors, motor disturbance, Parkinsonian-like extrapyramidal signs, and dysarthria. Also, WD may express itself as psychiatric manifestations; depression, anxiety or frank psychosis [2].

The diagnosis of WD is based on increased urinary copper excretion, reduced serum ceruloplasmin, detection of the Kayser-Fleischer ring in the cornea by slit lamp examination and liver biopsy findings [3]. Magnetic resonance imaging (MRI) findings in a WD range from normal

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to symmetric areas of T1-hyperintensity involving both basal ganglia (BG), thalami, external capsules, pons and midbrain [4]. Uncommonly, T2-weighted confluent hyperintensities in the subcortical white matter and cortical gray matter of parietal, temporal and frontal lobes may also be found in WD [5]. In contrast to conventional MRI, magnetic resonance spectroscopy (MRS) could provide information on the neuronal/axonal viability, cellular membrane status, and cellular energetics [6]. These advantages make MRS a very satisfactory technique that monitors in vivo metabolic variations caused by a certain disease and follows up after therapy [7]. The prominent resonances obtained on MRS in normal brains include N-acetyl aspartate (NAA), choline-containing compounds (Cho), creatine (Cr), and lactate. NAA is an important indicator of neuronal dysfunction. Abnormalities of neuronal structures as reduced neuronal viability leading to a reduction in NAA level. The choline-containing compound is a component of the phospholipids metabolism of cell membranes and contemplates membrane turnover [8]. Therefore, decreased Cho mostly represents decreased cell membrane synthesis and/or a decreased number of cells [9].

Several previous studies have been performed to deliberate the diagnostic role of MRS in WD. However, we could not find any studies that analyzed these changes in a pediatric population. Thus, we designed this prospective study to clarify the role of brain MRS in detecting early metabolite abnormalities in children with WD.

2. Methods

2.1. Study design

This prospective hospital-based group matched case-control study was conducted during the period from April 2016 to August 2018. We analyzed the metabolite changes in the brain of children with WD by the use of MRS and compared the results to those healthy controls to assess the ability of brain MRS in detecting early metabolite abnormalities in children with WD.

2.2. Ethical considerations

The institutional review board approved the study. All parents or guardians of the patients and controls were informed about the study, and they provided a written informed consent. The study was performed in keeping with the ethical principles of the Declaration of Helsinki.

2.3. Study population

The study included 26 children who were newly diagnosed with clinically definite WD, including 14 males and 12 females. Their ages ranged from 5 to 17 years (mean age = 10.8 ± 1.9 years). The diagnosis of WD was based on the combination of the followings: neurological symptoms, the presence of Kayser-Fleischer ring, low-level serum ceruloplasmin (< 20 mg/dl), increased excretion of urinary copper (> 100 μg/24 h), and the characteristic findings in hepatic biopsies.

Inclusion criteria: Child with clinically definite isolated WD.

Exclusion criteria:

- Co-existence of chronic viral hepatitis, autoimmune hepatitis and other metabolic diseases of the liver.
- Images of non-diagnostic quality due to severe motion artefacts.

We also carefully selected 26 age- and sex-matched healthy controls (13 males, 13 females, mean age = 9.3 ± 1.2 years, range 7 to 15 years) with no family history of a near relative (parent, sibling) with WD and not using copper containing medications in the past 6 months. The healthy controls were recruited from the hospital population where they were evaluated for unrelated health conditions, which were subsequently found to be negative.

2.4. Patient assessment

A complete history was taken from the family members, including different presentations of the disease and treatment. Full clinical examination was performed for all children, including full abdominal, regional, and neurological examinations. Anthropometry was done for all children. Ordinary laboratory investigations, including C-reactive protein (CRP), reticulocytic count, erythrocyte sedimentation rate (ESR), complete blood count (CBC), liver function tests, hepatitis markers, and kidney function tests were done. Specific laboratory investigations, including copper concentration in 24 h-urine collection, serum ceruloplasmin level, copper concentration in 24 h-urine collection with D-penicillamine challenge test, and auto-antibodies panel [anti-smooth muscle antibodies (ASMA), anti-liver/kidney microsomal antibodies (ALKMA), and antinuclear antibody (ANA)] were done. Slit lamp examination for Kayser-Fleischer ring was done. True-cut liver biopsy with histopathological analysis was performed for patients only. Radiological examination, including abdominal ultrasound, conventional MRI and MRS were done.

2.5. MRI protocol

Both WD patients and control subjects were examined by conventional MRI and MRS on a 1.5 T MR Unit (Achieva-class IIa, Philips Medical Systems) using quadrature head coil with 16 independent channels.

2.5.1. Conventional MRI

The following sequences were performed: T1-weighted sagittal localizer (repetition time (TR) = 10 ms, echo time (TE) = 500 ms); axial T1 (TR = 450 ms, TE = 15 ms, field of view (FOV) = 22 cm, matrix = 256 × 128); axial T2 (TR = 6000, TE = 100 ms, FOV = 22 cm, thickness = 5 mm, matrix = 256 × 128); axial and sagittal FLAIR (fluid-attenuation inversion recovery) (TR = 800, TE = 147 ms, FOV = 22 cm, thickness = 5 mm, matrix = 256 × 128).

2.5.2. Magnetic resonance spectroscopy

A 10 × 20 × 10 mm volume of interest (VOI) was placed in the center of the right globus pallidus. Single voxel spectroscopy (SVS) was obtained by a point-resolved spectroscopy sequence (PRESS) technique. First: Axial, sagittal and coronal planes of the brain were done in T2WI: TR = 3658, TE = 100, FOV = 18 × 24 cm and matrix = 256 × 192. Then, MRS in long TE = 288, short TE = 31 and intermediate TE = 144 with TR = 2000 and spectral bw = 1000. Upon initiation of SVS and chemical shift imaging (CSI), water was suppressed automatically for all patients by using chemical shift selective sequence (CHESS) and magnetic shimming.

2.6. Image processing and analysis

All data were transferred to the workstation and processed using the spectral analysis method of Philips Medical Systems (Spectra View, Philips, The Netherlands). Data processing included the following steps: (1) Time domain apodization filtering to improve signal-to-noise ratio (SNR), (2) Time domain reference phase correction using the signal from unsuppressed water, (3) Time domain high-pass filtering to remove residual water in the spectrum of interest, (4) Fourier transform to convert from time domain to frequency domain, (5) Phase correction, (6) Baseline subtraction, (7) Curve fitting, (9) Peak selection and peak fitting, and (10) Finally, calculation of peak heights and ratios of each metabolite within an individual voxel was performed with automatic one-button processing. The number of peaks fitted included the chemical shift ranges: N-acetyl asparate (NAA) at 2.0 ppm; creatine/phosphocreatine (Cr) at 3.0 ppm; choline compounds (Cho) at 3.2 ppm;

myo-inositol (M-ins) at 3.5 ppm; glutamine–glutamate–GABA complex (Glx) between 2.1 and 2.5 ppm; lactate (Lac) 1.35 ppm; free lipids (Lip): wide resonance at 1.3 and 0.9 ppm.

All MRI data were independently reviewed by two consultant radiologists. Any discrepancies in interpretation were resolved by a third consultant radiologist. All the radiologists were blinded to any clinical information. Special attention was given to any changes in signal intensity of globus pallidus, caudate nucleus, putamen, pons, and midbrain. The signal intensity of various metabolite peaks was evaluated in every voxel by using the integral of each peak as a measure of its intensity. Using the line-fitting program of the MR unit (Picker viStar Scan Suite, Philips Medical Systems), the spectral peak of each metabolite were determined and interpreted qualitatively and quantitatively. Qualitative assessment was done by inspection of the peaks and detection of any peak changes in WD patient group compared to the healthy control group. Quantitative assessment represented the measured peak of each metabolite (NAA, Cho, and Cr) and metabolite ratios (NAA/Cho, NAA/Cr, and Cho/Cr).

2.7. Statistical analysis

All collected data were statistically analyzed using SPSS (Statistical program for social science) version 15. Parametric values were expressed as mean ± standard deviation. Non-parametric values were expressed as median and range.The median of two groups was tested by Mann Whitney test. Qualitative data were represented by their frequency and relative percentage.The chi-square test was used for testing the association of qualitative data. For all tests, $p < 0.05$ was considered statistically significant.

3. Results

3.1. Study population

Demographic and clinical data of patients and controls are presented in Table 1. The mean duration of the disease from the onset of symptom to the time of presentation was 1.9 ± 2.2 years (range: 4 months to 3 years). There was no difference between studied groups as regard age and gender. Eight cases (30.7%) had neuropsychiatric symptoms. These symptoms included abnormal behavior, bad school performance, and abnormal movements as chorea and tremors. Significant differences were detected between patients and control as regards the mean values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, albumin and alkaline phosphatase ($p < 0.05$). Regarding the specific investigations, the amount of urinary copper in patients was markedly greater than in controls ($p < 0.001$). Moreover, the levels of ceruloplasmin were lower in patients than its levels in controls ($p = 0.012$). As regards autoantibodies, no significant difference was detected between WD and control groups as regard ANA and ALKMA. However, a significant difference was detected as regard ASMA ($p = 0.01$).

3.2. MRI and MRS abnormalities

We successfully obtained spectra from the brain of all subjects using MRS. The time of the whole MRS examination using the SVS technique ranged from 30 to 40 min. We found an abnormal MRI signal intensity on axial T1-weighted images in eight patients in the form of increased signal intensity at both basal ganglia (Fig.1). The other 18 patients showed no MRI brain abnormalities. However, as regards MRS, we found a highly significant difference ($p < 0.001$) between WD patients and control groups in the mean values of NAA, Cho, and Cr. Additionally, we detected significant differences between WD patients and control groups as regard NAA/Cho, NAA/Cr, and Cho/Cr of right basal ganglia ($p < 0.05$) (Table 2). We did not find a significant difference between WD patients who had brain MRI abnormalities and those who

Table 1
Demographic, Clinical, and laboratory data of patients and controls.

	Patients group (n = 26)	Control group (n = 26)	P-value
Demographic data	10.8 ± 1.9	9.3 ± 1.2	0.15
Age (years), mean ± SD	14 (53.8%)	13 (50%)	0.29
Sex, n (%)	12 (46.2%)	13 (50%)	
Male			
Female			
Clinical data, n (%)	26 (100%)	NA	
Jaundice	6 (23.1%)	NA	
Ascites and edema	24 (92.3%)	NA	
Hepatomegaly and/or	2 (7.7%)	NA	
splenomegaly	8 (30.7%)	NA	
Hematuria			
Neuro-psychatric			
manifestation			
Laboratory data, median	2.6 (0.3-7.6)	0.6 (0.3-0.9)	0.002*
(range)	2 (0.1-3.2)	0.13 (0.01-0.2)	0.024*
Total Bilirubin (mg/dl)	90.1 (19-200)	14.8 (12-20)	< 0.001**
Direct Bilirubin (mg/dl)	123.4 (34-450)	17.1 (10-22)	0.02*
AST (IU/L)	2.7 (1.1-4.5)	4.3(3.9-5.1)	< 0.001**
ALT (IU/L /)	148.1 ± 62.6	52.3 ± 12.5	0.026*
Albumin (g/dl)	26.4 ± 6.3	21.3 ± 4.1	0.7
Alkaline phosphatase,	0.7 ± 0.2	0.75 ± 0.18	0.09
mean ± SD	16 (61.5%)	8 (30.8%)	0.016*
Urea (mg/dl) mean ± SD	11.7 (4.4-35.9)	21.2 (18.8-25)	0.012*
Creatinine (mg/dl),	2219.8 ± 227	11.5 ± 2.9	< 0.001**
mean ± SD	0 (0%)	0 (0%)	1
Anemia, n (%)	8 (30.8%)	0 (0%)	0.01*
Ceruloplasmin level(mg/dl)			
24-hour urine copper (μg/			
24 h.), mean ± SD			
ANA/ALKMA, n (%)			
ASMA, n (%)			

AST = Aspartate Aminotransferase; ALT = Alanine Aminotransferase; ANA = Antinuclear antibody; ALKMA = Anti-liver/kidney microsomal antibodies; ASMA = Anti-smooth muscle antibodies; *= Significant; **= highly significant.

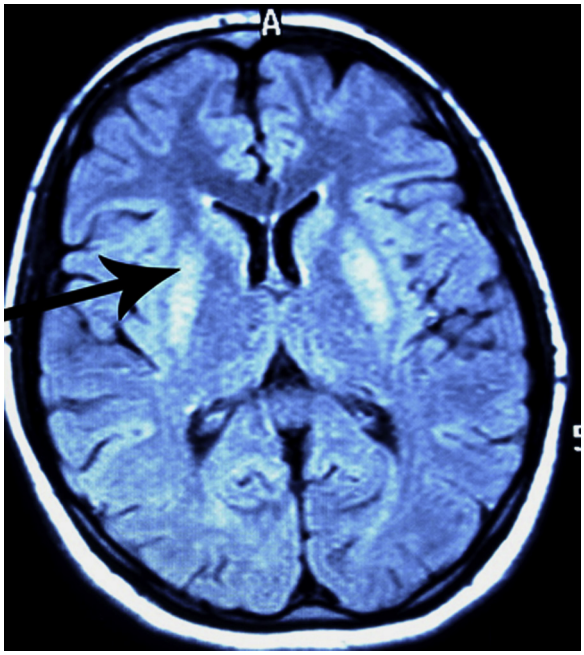

Fig. 1. Wilson's disease in a 10-year-old male patient with hepatosplenomegaly. T1-weighted axial MR image shows increase signal intensity in the right and left basal ganglia (black arrow).

Table 2
MRI and MRS results of patients and controls.

	Patient group (n = 26)	Control group (n = 26)	P-value
Brain MRI, n (%)	18 (69.3%)	26 (100%)	< 0.013*
Normal	8 (30.7%)	0 (0%)	< 0.001**
Abnormal	63.8 ± 9.6	97.6 ± 3.8	< 0.001**
MRS results, mean ± SD	46.7 ± 8.9	87.3 ± 4.7	< 0.001**
NAA	44 ± 10.1	81.9 ± 4.05	0.018*
Cho	1.92 ± 1.2	3.34 ± 0.55	0.002*
Cr	1.29 ± 0.7	2.46 ± 0.34	0.007*
NAA/Cho	0.78 ± 0.4	2 ± 0.13	
NAA/Cr			
Cho/Cr			

MRI = Magnetic resonance imaging; MRS = magnetic resonance spectroscopy; NAA = N-acetyl Aspartate; Cho = Choline; Cr = Creatine; *= Significant; **= highly significant.

Table 3
Relation between MRS results and type of presentation of WD.

	Patients with Hepatic presentations only (n = 18)	Patients with Hepatic and neurological presentations (n = 8)	P-value
NAA	72.7 ± 12.4	49.58 ± 21.6	0.03*
Cho	36.2 ± 10.6	37.6 ± 6.5	0.8
Cr	36.2 ± 7.4	30.5 ± 13.5	0.34
NAA/Cho	2.5 ± 1.1	0.98 ± 0.88	0.025*
NAA/Cr	1.59 ± 0.59	0.83 ± 0.6	0.046*
Cho/Cr	0.83 ± 0.5	0.68 ± 0.3	0.57

Date are mean ± SD.
WD = Wilsons' disease; MRS = magnetic resonance spectroscopy; NAA = N-acetyl Aspartate; Cho = Choline; Cr = Creatine; *= Significant; **= highly significant.

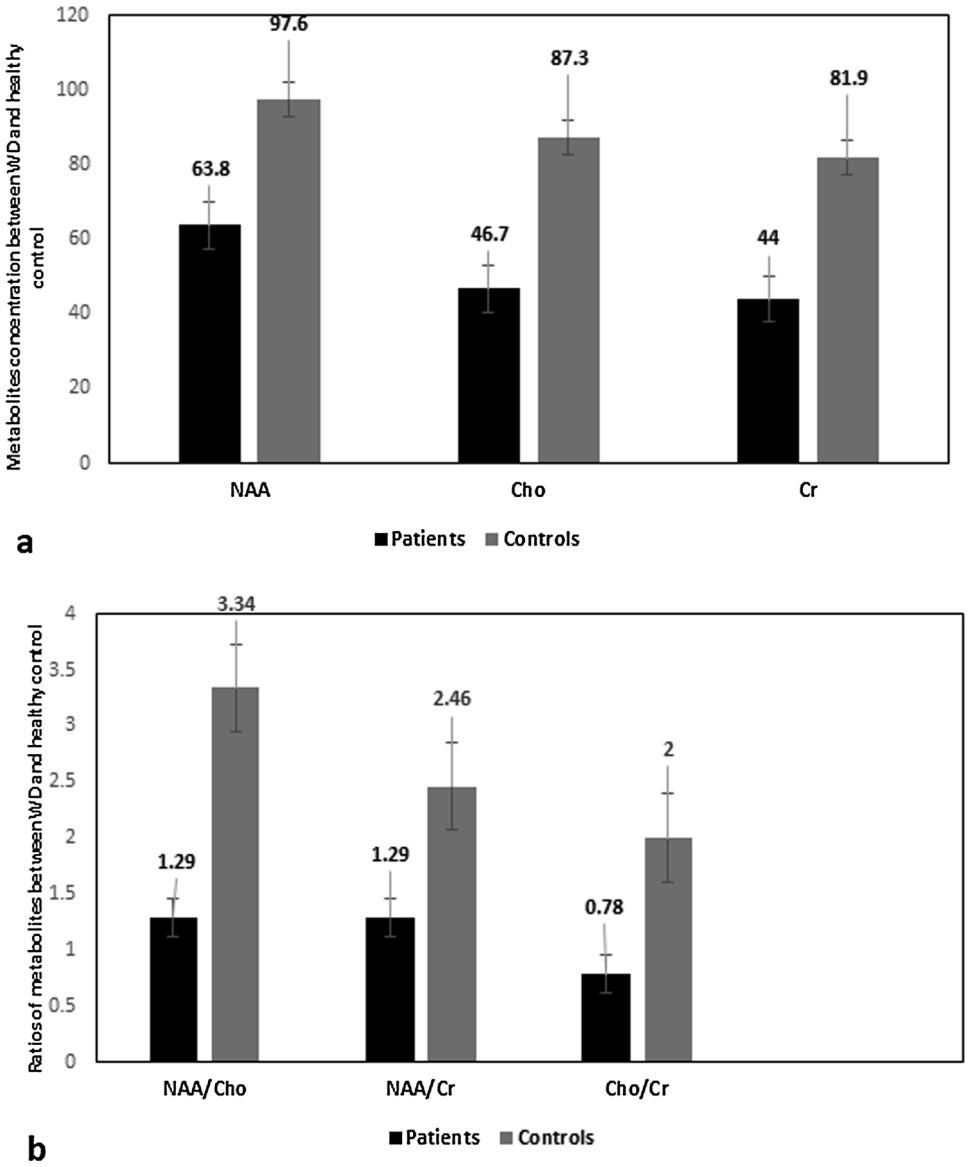

Fig. 2. Metabolite concentrations (a) and metabolite ratios (b) in patients with Wilson's disease compared to the control group. (Cho = choline, Cr = creatine, NAA = N-acetyl-aspartate). The values refer to the mean and error bars reflect standard errors.

Table 4
Relation between MRS results and complications of WD.

	Patients with no LCF (n = 20)	Patients with LCF (n = 6)	P-value
NAA	83.78 ± 15.1	48.5 ± 21.1	< 0.001**
Cho	59.6 ± 16.1	33.5 ± 6.7	0.04*
Cr	55.9 ± 14.1	30.1 ± 13.4	0.038*
NAA/Cho	2.71 ± 1	1.42 ± 0.58	0.047*
NAA/Cr	1.94 ± 0.67	0.89 ± 0.68	0.008*
Cho/Cr	1.37 ± 0.67	0.57 ± 0.29	0.019*

Date are mean ± SD.
WD = Wilsons' disease; MRS = magnetic resonance spectroscopy; LCF = liver cell failure; NAA = N-acetyl Aspartate; Cho = Choline; Cr = Creatine; *= Significant; **= highly significant.

had normal MRI as regard mean values of NAA, Cho, Cr, NAA/Cho, NAA/Cr, and Cho/Cr. The values of the metabolite peak intensities and ratios in the WD patients and healthy controls are illustrated in Fig. 2.

3.3. The relation of MRS and complications of WD

We detected significant reductions in NAA, as well as NAA/Cr, and NAA/Cho ratio in patients who had mixed neurological and hepatic diseases compared with patients who solely had hepatic disease without neurological disease (Table 3). Moreover, we found significant differences between patients without complications and those complicated with liver cell failure in the mean values of NAA, Cho, and Cr, as well as NAA/Cr, NAA/Cho and Cho/Cr ratios (Table 4 and Figs. 3 and 4).

4. Discussion

WD in children remains a diagnostic challenge due to its rather non-specific presentation and the requirement of a high index of clinical suspicion and extensive investigation to establish the diagnosis. Early recognition is vital as the severity of the disease can be modified by prompt medical intervention. Whilst brain MRI can be helpful in making the diagnosis of WD and in excluding other conditions, our results confirm previous studies showing that the majority of patients with WD demonstrate normal structural MR appearances. Although brain MRS imaging findings in adult WD have been described in considerable details, to the best of our knowledge, the use of MRS for the detection of brain metabolite abnormalities of WD in pediatric age group has not yet been described. In the present prospective comparative analysis, we analyze the metabolite changes in the brain of children with WD by the use of MRS and compare the results to those healthy controls to evaluate the ability of brain MRS in detecting early metabolite abnormalities in children with WD.

Our study showed that NAA, Cr and Cho, as well as NAA/Cho, NAA/Cr, and Cho/Cr ratios, are decreased in all WD patients in comparison to the control group. These results are consistent with the results of previous studies [10–14], which demonstrated low Cho and NAA in MRS of a patient with WD. As well as, Van Den Heuvel et al. [15] who studied 22 patients with neurological symptoms in WD and detected a severe reduction of Cho/Cr and NAA/Cr of the brain than in the healthy controls. On the other hand, our observation of Cho/Cr ratio was not similar to that of Lucato et al. [16] who found an increase in the Cho/Cr ratio in the basal ganglia of the patient group. However, they reported a significant decrease in NAA/Cr ratio. In addition, our collected data are opposing the findings of Kraft et al. [17]. This difference may be attributed to the non-identical TR used in obtaining the spectra.

As regards relation between MRS and severity of disease, our results showed significant decreases in the mean values of NAA, Cho, Cr, NAA/Cho, NAA/Cr and Cho/Cr in patients complicated with liver cell failure compared with patients without this complication. This may suggest that liver failure precedes and participates in neuronal damage. This result is in agreement with the result of Matsusue et al. [18]. Furthermore, there was a severe reduction in the brain metabolites (NAA, NAA/Cr, and NAA/Cho ratio) in patients who had mixed neurological and hepatic diseases compared with patients who solely had hepatic disease without neurological disease. These findings may reflect the long-standing status, which destroys basal ganglia cells and produces such metabolites or they may be due to the synergistic action of both neurological and hepatic diseases.

In our study, eight patients who had neurological symptoms displayed MRI abnormalities in the form of increased signal intensity in both basal ganglia on axial T1-weighted images. We did not find a significant difference between WD patients who had brain MRI abnormalities and those who had normal MRI as regard mean values of NAA, Cho, Cr, NAA/Cho, NAA/Cr, and Cho/Cr. This result is parallel to the result of Lucato et al. [16] who detected decrease NAA/Cr ratio in regions with a normal image on MRI. This demonstrates the global involvement of the brain in WD and suggests that the biochemical variations noted in WD do not necessary concomitant to visible structural alterations on MRI finding. Therefore, MRS can be more sensitive than MRI in early detection of neurological changes in patients with WD who might have normal MR imaging.

One strength of our study, its prospective nature, thereby avoiding the selection bias of retrospective studies. However, our study had some

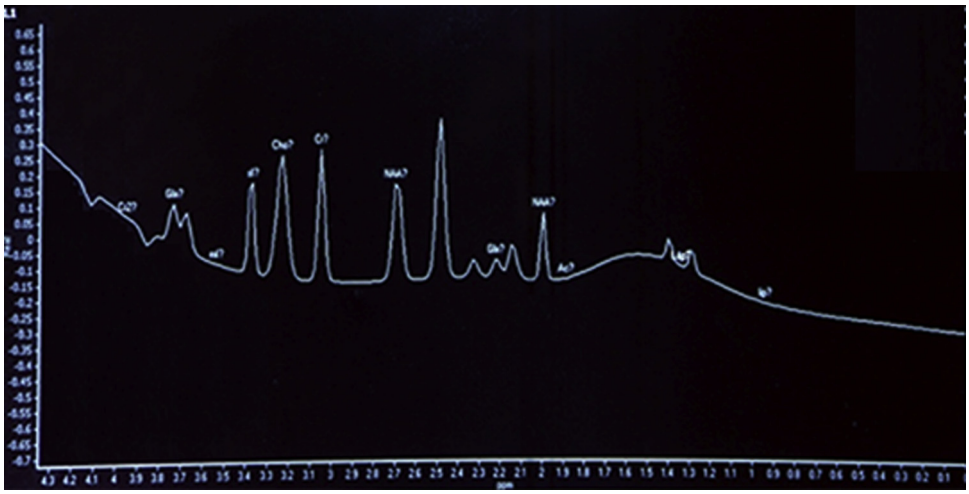

Fig. 3. Wilson's disease in a 15-year-old female patient with hepatomegaly and chorea. MRS from right basal ganglia shows reduced NAA, Cho and Cr (reduction of the NAA peak relative to the Cr peak).

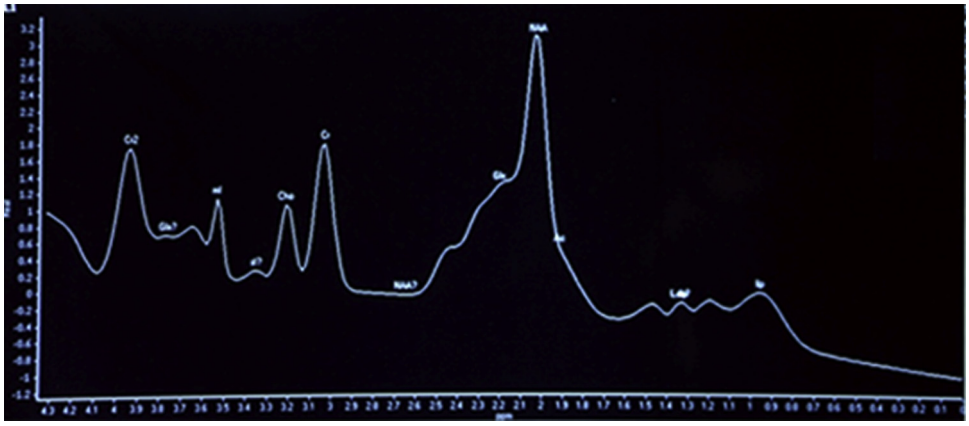

Fig. 4. Wilson's disease in a 12-year-old male patient complicated with liver cell failure. MRS from right basal ganglia shows reduced NAA, Cho, and Cr.

limitations. First, the small sample size. Second, we did not include the effect of drugs on the basal ganglia peak metabolites concentration to monitor the response to medications. Third, no test-retest reproducibility data. Fourth, we could not measure the M-Ins/Cr ratio, as M-Ins was not optimum in our MRS examinations. Finally, all examinations were performed on 1.5 T MRI

5. Conclusion

Brain MRS is a more sensitive diagnostic and prognostic tool than conventional MRI in detecting early changes in brains in children with WD, as MRS can detect metabolite abnormalities affecting the brain before structural changes become visible on MRI.

Conflict of interest

The authors affirm that they have no conflict of interest.

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Ethical approval

Institutional review board approval was gained.

Informed consent

Written informed consent was attained from all patients.

Methodology

- Prospective
- Diagnostic study.
- Achieved at single institution.

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