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Functional Brain MRI as a Diagnostic Tool for Detecting Neurological Changes in Children with Wilson's Disease

Sameh Abokoura[1], Ahmed Elkholy[1], Enas Mohammed Korayem[1], Nermin M. Adawy[2], Hazem Omar*[1]
Departments of [1]Diagnostic and Intervention Radiology,
[2]Pediatric Hepatology, Gastroenterology, Nutrition, National Liver Institute, Menoufia University, Egypt
***Corresponding author:** Hazem Omar, **Mobile:** (+20) 01228229522, **E-mail:** hazemomar2020@gmail.com

ABSTRACT

Background: Wilson's disease (WD) is a rare genetic disorder marked by copper buildup, causing hepatic and neurological issues. **Objective:** This study aimed to thoroughly evaluate clinical, biochemical, and neuroimaging aspects of WD, highlighting the diagnostic potential of magnetic resonance spectroscopy (MRS) and diffusion-weighted imaging (DWI) for detecting neurological involvement.

Patients and methods: The study included 40 children divided into two groups: 20 healthy children (group 1) and 20 with Wilson's disease (group 2). Various MRI techniques, including T1, T2, FLAIR, diffusion imaging, and MRS, were used. For WD patients, comprehensive clinical examinations, lab tests, and MRS were performed to understand their neurochemical profile. The analysis involved assessing clinical features (hepatomegaly prevalence) and lab parameters (24-hour urine copper excretion, albumin, alkaline phosphatase, bilirubin, and ceruloplasmin). MRS provided insights into metabolites like N-acetyl aspartate (NAA), choline (Cho), and creatine (Cr). The diagnostic accuracy of neurochemical ratios (NAA/Cr, NAA/Cho, Cho/Cr) for predicting neurological manifestations was evaluated, alongside conventional brain MRI and diffusion MRI for neurological status assessment.

Results: The study found hepatomegaly in 70% of WD patients, a significant range of 24-hour urine copper excretion was from 10 to 4699 µg/24h and a median of 122.5 µg/24h (IQR=27.5-309) and distinct neurochemical shifts in NAA, Cho, and Cr. Brain MRI detected neurological abnormalities in 80% of patients. Cho showed promise (P-value: 0.038) in predicting neurological issues, while caution was urged against relying solely on Cr due to lower specificity (p-value: 0.777). The NAA/Cr ratio proved a robust predictor (P-value: 0.006), surpassing NAA/Cho (P-value: 0.925).

Conclusion: Our study underscored the clinical significance of hepatomegaly, elevated 24-hour urine copper excretion, and distinctive neurochemical shifts in WD. Neuroimaging findings, especially using diffusion-weighted imaging, contributed valuable insights into neurological manifestations. The diagnostic potential of MRS-derived ratios, notably the NAA/Cr ratio, hold promise for predicting neurological involvement in Wilson's disease.

Keywords: Wilson's disease, Magnetic resonance spectroscopy, Neurological manifestations, Diffusion MRI.

INTRODUCTION

Wilson's disease (WD) is a rare autosomal recessive disorder characterized by abnormal copper metabolism, leading to copper accumulation in various organs, including the liver, brain, and kidneys. The global incidence of WD is approximately 1 in 50,000 live births **(1).** Clinically, WD manifests with hepatic and Neurological features, ranging from biochemical abnormalities to acute hepatic failure and neurological symptoms such as tremors, motor disturbances, and psychiatric manifestations like depression and psychosis **(2).** Diagnostic criteria for WD include increased urinary copper excretion, reduced serum ceruloplasmin, the presence of the Kayser-Fleischer ring in the cornea, and liver biopsy findings. Conventional magnetic resonance imaging (MRI) reveals diverse findings, from T1-hypointensity in basal ganglia and other regions to T2-hyperintensity. Additionally, subcortical white matter and cortical gray matter abnormalities may occur **(3).** In contrast to conventional MRI, functional MRI techniques, specifically magnetic resonance spectroscopy (MRS) and diffusion-weighted imaging (DWI), offer insights into early neurological changes before clinical symptoms manifest. MRS provides information on neuronal viability, cellular membrane status, and energetics, making it a valuable tool for monitoring metabolic variations and treatment response **(4).** NAA, choline-containing compounds (Cho), creatine (Cr), and lactate are key resonances in MRS, reflecting neuronal dysfunction and membrane turnover. Reduced Cho signifies decreased cell membrane synthesis and/or cell number **(5).**

Diffusion MRI, particularly apparent diffusion coefficient (ADC) maps, provides detailed information about tissue integrity. It reflects molecular water motion, with low ADC values indicating restricted diffusion in conditions like cytotoxic edema, and high ADC values suggesting increased water mobility in vasogenic edema **(6).** Our study investigated WD by incorporating conventional magnetic resonance imaging (MRI) sequences, including T1, T2, and FLAIR, alongside advanced functional MRI techniques such as magnetic resonance spectroscopy (MRS) and diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) mapping. By integrating these comprehensive imaging modalities, we aimed to enhance the characterization of early metabolite abnormalities in children with WD.

PATIENTS AND METHODS

Study design and participants: This prospective cohort study was conducted at the Diagnostic Medical Imaging and Interventional Radiology Department of the National Liver Institute Hospital, Menoufia University.The study included two groups: Group I

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comprised 20 healthy controls, and group II that consisted of 20 individuals diagnosed with WD. WD was identified through clinical evaluation, including history, physical examination, and specific diagnostic criteria such as increased urinary copper excretion, reduced serum ceruloplasmin, detection of Kayser-Fleischer ring, and liver biopsy findings.

Inclusion criteria: The study focused on children clinically diagnosed with Wilson disease.

Exclusion criteria: The study excluded those with chronic viral hepatitis, autoimmune hepatitis, other metabolic liver diseases, poor imaging quality due to motion artifacts, and contraindications for MRI (e.g., pacemakers & cochlear implants).

Evaluation methods: All participants underwent a comprehensive assessment, including full history taking, physical examination, and laboratory investigations such as liver function tests (SGOT, SGPT Albumin, and Bilirubin), serum urine copper, ceruloplasmin, and alkaline phosphatase). Radiological imaging, specifically by incorporating conventional magnetic resonance imaging (MRI) sequences, including T1, T2, and FLAIR. Alongside advanced functional MRI techniques such as magnetic resonance spectroscopy (MRS) and diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) mapping. These investigations were performed within a week before MR scanning for all participants.

Radiological imaging techniques:

1. MRI examination:

All subjects were studied on clinical Optima MRI 450W GEM 1.5 T Elite MRI system (GE Healthcare, Milwaukee, WI, USA) with T1, T2, and Flair weighted image sequences acquired.

2. MRS evaluation:

MRS was employed for metabolite quantification in the brain, particularly in the basal ganglia. Multi-voxel MRS utilized a stimulated-echo acquisition mode sequence (STEAM) with the following parameters: TR= 1102 ms; TE= 144 ms. Metabolites (choline, creatine, and N-acetyl aspartate) were identified and quantified. Integrals of Cho, NAA, and Cr were calculated, along with ratios (NAA/Cho, Cho/Cr, and NAA/Cr).

3. Diffusion-weighted MR imaging: Diffusion-weighted MR imaging was conducted by using single-shot spin-echo echoplanar imaging. The diffusion weighting gradients were applied on each of the three physical axes x, y, and z with b factor values of 1000 mm^2/s. The scanning parameters were TR/TE= 4000/1000 ms, number of averages =1, FOV= 240 x 240 mm, slice thickness = 5 mm, interslice gap = 0.5 mm, and matrix = 96 x 128 pixels. After the application of diffusion-weighted sequences, we obtained a set of images corresponding to the P-value applied. The available software automatically calculated the apparent diffusion coefficient (ADC) map. The data acquisition time for the diffusion-weighted MR images was 1 minute**.** The mean ADC was determined in manually drawn regions of interest placed in basal ganglia. The ADC value was calculated according to the following formula: ADC= (InSb-InSb1)/ (b2-b1), where In is the natural log, and Sb1 and Sb2 are the signal intensities in the ROI placed on sections corresponding to the b value. The ADC value is automatically calculated in 10^{-3} mm^2/s**.**

Data Analysis

The collected data were analyzed using SPSS software (version 26) on an IBM-compatible computer. Descriptive statistics expressed qualitative data as numbers and percentages, and quantitative data as mean ± SD or median (IQR) depending on the distribution. Analytic statistics included the Pearson Chi-squared test, Fisher Exact test, Student t-test, Mann-Whitney U test, and Receiver Operator Characteristic (ROC) analysis. Sensitivity and specificity were assessed, and statistical significance was defined at a p-value ≤ 0.05 in all analyses.

RESULTS

Sociodemographic Characteristics:

The sociodemographic profile of the study participants (n=40) revealed that among the 20 patients, 50% were males and 50% were females, while in the control group (20 individuals) 40% were males and 60% were females. The comparison did not show a significant difference in gender distribution between the patient and control groups ($\chi2=0.404$, p=0.525), indicating a balanced representation. Regarding age, the mean age in the patient group was 12.95 ± 3.05 years ranging from 7 to 17 years, while the control group had a mean age of 14.80 ± 2.80 ranging from 7 to 17 years. The age difference did not reach statistical significance (t=1.845, p=0.073), suggesting a comparable age distribution between patients and controls (Table 1).

Table (1): Sociodemographic characteristics of the studied participants (n=40)

	Variable	Patients (n=20)		Controls (n=20)		Test of significance	p-value
		No.	%	No.	%		
Sex	Male	10	50	8	40	$\chi2=0.404$	
	Female	10	50	12	60		0.525
Age (Years)	Mean ±SD	12.95 ±3.05		14.80 ±2.80		t=1.845	
	Range (Min-Max)	7-17		7-17			0.073

SD: Standard deviation, $\chi2$: Chi-squared test, t: Student t-test

Clinical Findings:

In the patient group (n=20), hepatomegaly was observed in 70%, while 30% showed no evidence of hepatomegaly. Splenomegaly was present in 35%, with 65% showed absence of splenomegaly. Edema was present in only 5%, while 95% did not exhibit edema. Ascites was observed in 10%, and 90% did not have ascites. Encephalopathy was present in 5%, and 95% showed no signs of encephalopathy. Neurological manifestations were noted in 20%, and 80% did not exhibit these manifestations (Table 2).

Table (2): Clinical findings in studied patients (n=20)

Variable	No of studied patients =20	
	No.	**%**
Hepatomegaly		
Present	14	70
Absent	6	30
Splenomegaly		
Present	7	35
Absent	13	65
Edema		
Present	1	5
Absent	19	95
Ascites		
Present	2	10
Absent	18	90
Encephalopathy		
Present	1	5
Absent	19	95
Neurological manifestation	4	20
Present	16	80
Absent		

Laboratory findings: Among the studied patients (n=20), the range of 24-hour urine copper level was 10 to 4699 µg/24h and a median of 122.5 µg/24h (IQR=27.5-309). The mean albumin level was 3.37 ± 0.83 g/dl. The ranging of alkaline phosphatase level was from 10 to 450 U/L, with a median of 58.5 U/L (IQR=34-169.25). The range bilirubin level was a from 0.3 to 54 mg/dl, and a median of 1.25 mg/dl (IQR=1-1.925). The range of ceruloplasmin level was from 1 to 19 mg/dl, and the median was 5.5 mg/dl (IQR=2.75-9.25) (Table 3).

Table (3): Laboratory findings in studied patients (n=20)

Variable	No of studied patients =20
24 hr urine copper (µg/24h) Range (Min-Max) Median (IQR)	 10-4699 122.5 (27.5-309)
Albumin (g/dl) Mean ±SD	 3.37 ±0.83
Alkaline phosphatase Range (Min-Max) Median (IQR)	 10-450 58.5 (34-169.25)
Bilirubin (mg/dl) Range (Min-Max) Median (IQR)	 0.3-54 1.25 (1-1.925)
Ceruloplasmin (mg/dl) Range (Min-Max) Median (IQR)	 1-19 5.5 (2.75-9.25)

Range and Median (IQR): non parametric test,
SD: Standard deviation, IQR: Interquartile range.

Magnetic Resonance Spectroscopy (MRS) Findings: In the evaluation of metabolite concentrations using MRS, significant differences were observed between patients with Wilson disease (n=20) and healthy controls (n=20). Notably, the mean N-acetyl aspartate (NAA) levels were significantly lower in patients (109.47 ± 40.31) compared to controls (203.09 ± 34.19), with a p-value < 0.001. Similarly, choline (Cho) levels were reduced in patients (67.56 ± 22.50) compared to controls (97.14 ± 10.25), showing statistical significance with a p-value < 0.001. Creatine (Cr) levels exhibited a significant difference as well, with patients (68.87 ± 21.60) having lower levels than controls (80.65 ± 15.15), and a p = 0.037. Other ratios were calculated to provide insights into the metabolic profile. The NAA/Cr ratio in patients (1.71 ± 0.62) was significantly lower than in controls (2.59 ± 0.59) with a p < 0.001. The NAA/Cho ratio was also reduced in patients (1.69 ± 0.47) compared to controls (2.12 ± 0.42), with a p = 0.004. Additionally, the Cho/Cr ratio was lower in patients (1.04 ± 0.31) than in controls (1.24 ± 0.22), demonstrating statistical significance with a p-value of 0.030 (Table 4).

Table (4): MRS findings in studied participants (n=40)

Variable	Patients (n=20)	Controls (n=20)	Test of significance	p-value
NAA				
Mean ±SD	109.47 ±40.31	203.09 ±34.19	U=5.116	**<0.001***
Range (Min-Max)	38.57-156.73	102.3-234		
Median (IQR)	119.13(75.3-144.21)	220 (174.7-226)		
Cho				
Mean ±SD	67.56 ±22.50	97.14±10.25	t=5.349	**<0.001***
Range (Min-Max)	19.77-108.26	75.22-120.86		
Median (IQR)	70.83 (57.09-85.25)	99.72 (96.52-101.98)		
Cr				
Mean ±SD	68.87 ±21.60	80.65±15.15	t=2.166	**0.037***
Range (Min-Max)	26.33-113.7	52.41-114.85		
Median (IQR)	65.54 (54.06-86.37)	77.71 (70.65-89.49)		
NAA/Cr				
Mean ±SD	1.71 ±0.62	2.59±0.59	U=3.842	**<0.001***
Range (Min-Max)	0.34-2.77	1.25-3.72		
Median (IQR)	1.77 (1.53-1.97)	2.72 (2.22-3.09)		
NAA/Cho				
Mean ±SD	1.69 ±0.47	2.12±0.42	t=3.082	**0.004***
Range (Min-Max)	0.56-2.52	0.85-2.9		
Median (IQR)	1.74 (1.28-2)	2.20 (1.86-2.33)		
Cho/Cr				
Mean ±SD	1.04 ±0.31	1.24±0.22	t=2.258	**0.030***
Range (Min-Max)	0.17-1.54	0.80-1.57		
Median (IQR)	1.06 (0.94-1.24)	1.26 (1.07-1.40)		

Range and Median (IQR): non parametric test, *: Statistically significant, NS: Non-significant, SD: Standard deviation, IQR: Interquartile range, t: Student t-test, U: Mann-Whitney test, MRS: Magnetic resonance spectroscopy, NAA: N-acetyl aspartate, Cho: Choline, Cr: Creatine.

Brain MRI findings:

The analysis of brain MRI findings in participants (n=40) demonstrated that 80% of patients had abnormal MRI results, while all controls exhibited normal MRI findings. This discrepancy was statistically significant ($\chi 2=4.44$, p=0.035), indicating a substantial association between Wilson's disease and abnormal brain MRI results (Table 5).

Table (5): Brain MRI in studied participants (n=40)

Brain MRI	Patients (n=20)		Controls (n=20)	
	No	%	No	%
Normal	16	80	20	100
Abnormal	4	20	0	0
Test of significance	χ^2 =4.44			
p-value	**0.035***			

*: Statistically significant, χ^2: Chi-squared test, MRI: Magnetic resonance imaging

Diffusion MRI Findings and Neurological Manifestations: Among the patients, DWI results were further analyzed in relation to the presence of neurological manifestations. The apparent diffusion coefficient (ADC) values showed no significant difference between patients with positive (n=4) and negative (n=16) neurological manifestations (0.93 ± 0.16 vs. 0.93 ± 0.16, p = 0.968). Similarly, the DWI results indicated no significant difference in the presence of restricted or non-restricted diffusion between these two groups (p = 0.509). Regarding T1-weighted and T2/FLAIR MRI findings, no significant differences were observed between patients with positive and negative neurological manifestations indicating uniformity in these imaging parameters across the patient cohort (Table 6).

Table (6): Diffusion MRI findings according to Neurological manifestations in studied patients (n=20)

Variable	Positive (n=4)		Negative (n=16)		Test of significance	p-value
	No.	%	No.	%		
ADC Mean ±SD Range (Min-Max)	0.93 ±0.16 0.73-1.1		0.93 ±0.16 0.75-1.3		t=0.043	0.968
DWI Restricted Non-restricted **(free)**	1 3	25 75	2 14	12.5 87.5	FE	0.509
T1 Normal intensity Hyperintense	4 0	100 0	14 2	87.5 12.5	FE	1.000
T2/ FLAIR Normal intensity Hyperintense	3 1	75 25	14 2	87.5 12.5	FE	0.509

SD: Standard deviation, FE: Fisher exact test, t: Student t-test, ADC: Apparent diffusion coefficient, DWI: Diffusion-weighted imaging, FLAIR: Fluid attenuated inversion recovery.

Diagnostic Accuracy for Predictors of Neurological Manifestation:

The diagnostic accuracy of metabolite ratios as predictors of neurological manifestations in studied patients (n=20) with Wilson's disease was evaluated. Notably, NAA demonstrated exceptional diagnostic performance, with an area under the curve (AUC) of 1.000 (p=0.002), a cutoff point of 71.39, 100% sensitivity, and 100% specificity. Choline (Cho) also exhibited significant predictive capability, with an AUC of 0.844 (p=0.038), a cutoff point of 44.96, 75% sensitivity, and 100% specificity. Creatine (Cr) showed limited discriminatory power with an AUC of 0.547 (p=0.777), a cutoff point of 66.83, 75% sensitivity, and 50% specificity. The NAA/Cr ratio demonstrated strong diagnostic accuracy with an AUC of 0.953 (p=0.006), a cutoff point of 1.60, 100% sensitivity, and 88% specificity. In contrast, NAA/Cho and Cho/Cr exhibited lower discriminatory ability with AUCs of 0.516 (p=0.925) and 0.734 (p=0.156), respectively, alongside varying sensitivity and specificity values (Table 7).

Table (7): Diagnostic accuracy for predictors of Neurological manifestation in studied patients

	NAA	CHO	CR	NAA/CHO	NAA/CR	CHO/CR
AUC	1.000	0.844	0.547	0.516	0.953	0.734
p-value	**0.002***	**0.038***	0.777	0.925	**0.006***	0.156
95% CI	1.000-1.000	0.567-1.000	0.173-0.921	0.164-0.868	0.862-1.000	0.345-1.000
Cutoff point	71.39	44.96	66.83	1.67	1.60	0.84
Sensitivity	100%	75 %	75 %	50%	100%	75%
Specificity	100%	100 %	50 %	63%	88%	94%

CASES

Case (1)

An 8-year-old male patient with no hepatomegaly. MRS from left basal ganglia showed reduced NAA, CHO, and Cr, reduction of NAA/CHO, NAA/ CR, and CHO/CR ratios (A) , and no areas of diffusion restriction at DWI and ADC. (B)

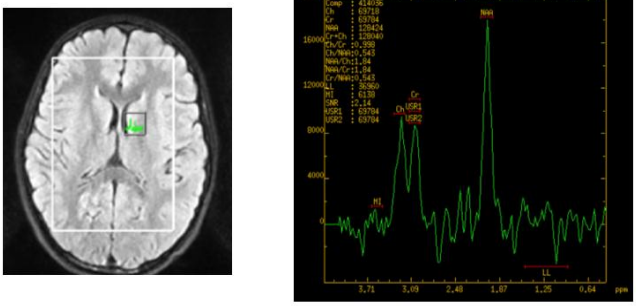


Image A : MRS from left basal ganglia

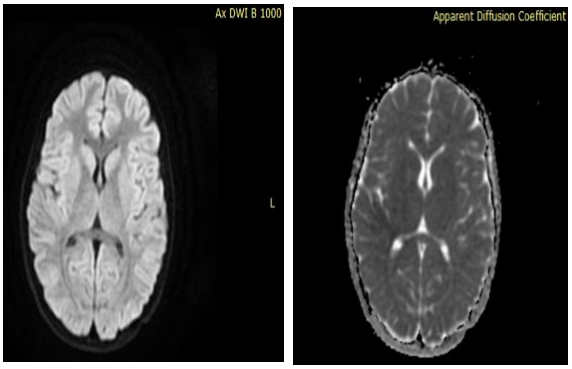


Image B : DWI and ADC

Case (2)

A 17-year-old female patient with Wilson's disease, hepatosplenomegaly, and neurological manifestations as fine tremors showed increased signal of basal ganglia at FLAIR& T2 weighted axial MR images,(Arrows)(A) MRS from left basal ganglia showed reduced NAA, CHO, and Cr, reduction NAA/CHO, and NAA/ CR ratios (B) areas of facilitated diffusion at basal ganglia on DWI and ADC.(C)

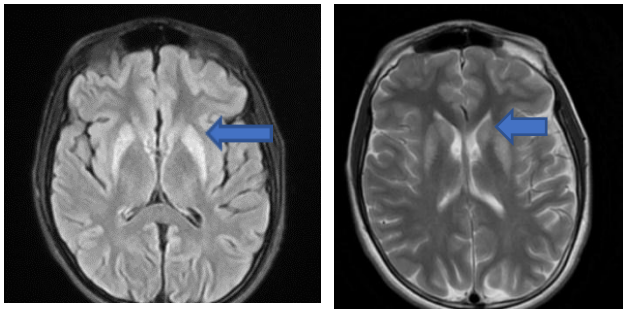


Image A : FLAIR and T2 weighted images

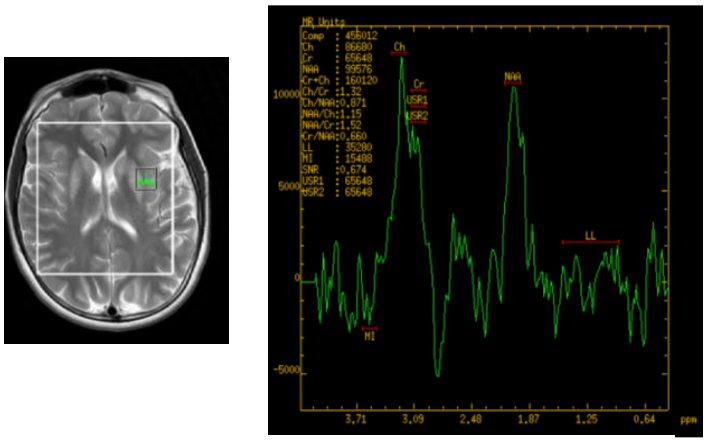


Image B:MRS from left basal

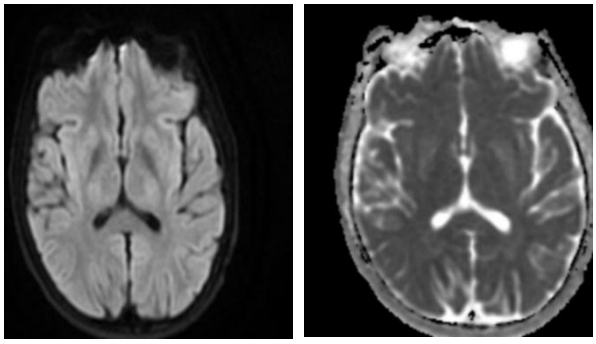


Image C : DWI and ADC

Case (3)

A 15-year-old female with Wilson's disease displayed hepatosplenomegaly without Neurological changes. T2 WI of basal ganglia shows increased signals(A). Magnetic resonance spectroscopy (MRS) revealed a reduction in N-acetylaspartate (NAA) and NAA/CHO, as well as NAA/CR, indicating potential neurological alterations. However, the CHO/CR ratio remained within normal limits, and signals that confirmed neurological involvement. (B) while basal ganglia showed facilitated diffusion (C)

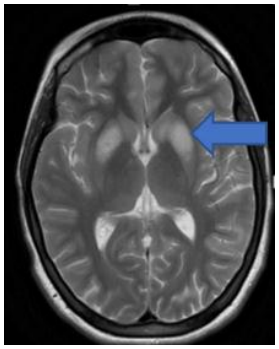
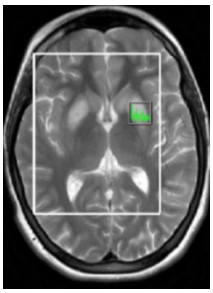


Image A :T2 weighted image



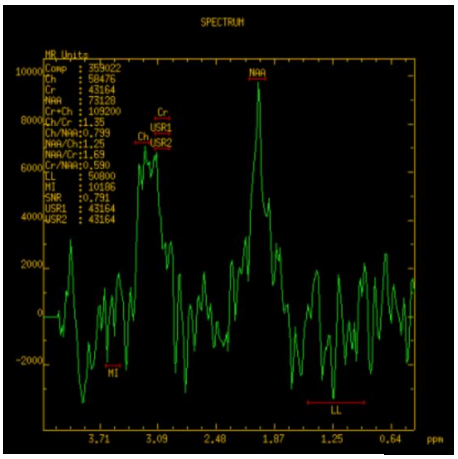


Image B: MRS from left basal

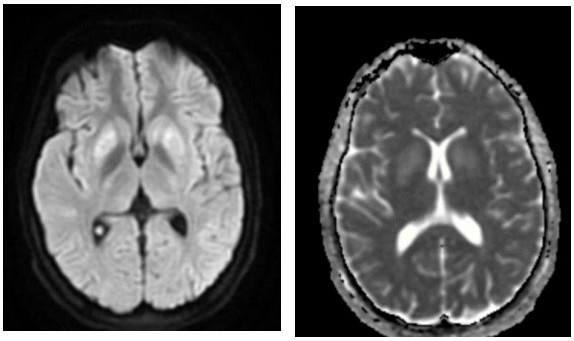


Image C : DWI and ADC

DISCUSSION

Wilson's disease (WD) is a rare autosomal recessive disorder characterized by abnormal copper metabolism, resulting in copper accumulation in various organs, leading to hepatic and neurological manifestations. The global incidence of WD is approximately 1 in 50,000 live births, highlighting its rarity and clinical significance [(1)]. Clinically, WD presents a spectrum of features, ranging from biochemical abnormalities to acute hepatic failure and diverse neurological symptoms, making early detection crucial for effective management [(2)].

Our study delves into the diagnostic potential of advanced functional MRI techniques, specifically multivoxel magnetic resonance spectroscopy (MRS) and diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) mapping, to identify early metabolite abnormalities in pediatric patients with Wilson's disease. Emphasizing the significance of early detection in pediatrics, our research aimed to elucidate the role of these techniques in offering a comprehensive diagnostic approach to this complex disorder. Multivoxel MRS enables a detailed examination of metabolite abnormalities, serving as early markers for timely therapeutic interventions and improved disease management. The integration of DWI with ADC mapping enhances the diagnostic utility, providing insights into tissue microstructure changes crucial for implementing targeted interventions in pediatric WD patients [(7)].

Our study, with 40 participants, found no statistically significant differences in sociodemographic characteristics between Wilson's disease (WD) patients (n=20) and controls (n=20) for sex (χ^2=0.404, p=0.525) and age (t=1.845, p=0.073). Hepatomegaly emerged as the predominant clinical finding in 70% of WD cases. This is aligning with **Mulroy *et al.*** [(8)] highlighting its significance. Biochemical analysis revealed elevated 24-hour urine copper excretion [a range of 10 to 4699 µg/24h and a median of 122.5 µg/24h (IQR=27.5-309)] and provided insights into hepatic and metabolic aspects, which is consistent with **Lafhal *et al.*** [(9)].

Our study integrated magnetic resonance spectroscopy (MRS) to unveil distinctive neurochemical variations in individuals with WD when

compared to controls. The observed decrease in N-acetyl aspartate (NAA) and alterations in Cho and Cr levels, coupled with shifts in NAA/Cr and NAA/Cho ratios, underscored the profound neurological implications of WD. This substantiated the efficacy of MRS as a valuable tool for assessing neurochemical changes, which is aligning with the findings of **Kasztelan-Szczerbinska and Cichoz-Lach [10].** Our findings further enriched the existing body of literature concerning the diagnostic potential of neuroimaging and biochemical markers in the context of WD.

Our study delved into brain magnetic resonance imaging (MRI), uncovering a notable contrast in abnormalities between WD patients and controls, mirroring findings in 80% of WD patients. This is in line with the observations made by **Shribman *et al.* [11].** Despite this, an examination of diffusion MRI parameters, encompassing apparent diffusion coefficient (ADC) values and diffusion-weighted imaging (DWI) characteristics, failed to unveil statistically significant distinctions between WD patients and controls. This underscores the intricate nature of neurological changes in WD, a perspective that is supported by previous studies by **Tae *et al.* [12].**

Our study delved into the correlation between diffusion MRI results and neurological manifestations in individuals with WD and revealed no noteworthy distinctions in apparent diffusion coefficient (ADC) values and diffusion-weighted imaging (DWI) findings, or T1 and T2/FLAIR intensity characteristics between patients with and without neurological manifestations. Despite the absence of significant disparities, the need for additional exploration using larger cohorts and comprehensive protocols is evident to comprehend the nuanced relationship, as suggested by **Wang *et al.* [13].**

In contrast, our study demonstrated the exceptional diagnostic accuracy of N-acetyl aspartate (NAA) in predicting neurological manifestations in WD patients. NAA levels, measured by MRS, exhibited perfect discrimination with 100% sensitivity and specificity, which is aligning with **Page *et al.* [14]** who emphasized the value of MRS in assessing neurochemical alterations in WD patients with neurological symptoms. Identifying NAA as a potential biomarker in diagnosing and monitoring neurological involvement in WD underscored its clinical utility and highlighted the potential role of neurochemical markers, particularly NAA, in evaluating neurological involvement in WD.

Our study investigated the predictive value of choline (Cho) and creatine (Cr) levels for neurological manifestations in individuals with Wilson's disease (WD), yielding promising results. Cho exhibited a reasonably robust discriminatory capability (AUC=0.844, sensitivity=75%, specificity=100%), suggesting its potential as a useful marker for identifying neurological involvement in WD patients, as supported by **Derbyshire and Obeid [15].** Conversely, caution is advised in relying solely on Cr as a predictor, given its AUC of 0.547 and lower specificity, as highlighted by findings from **Guenter *et al.* [16].**

Our study examined neurochemical ratios, specifically N-acetyl aspartate to creatine (NAA/Cr) and N-acetyl aspartate to choline (NAA/Cho), as potential predictors of neurological manifestations in Wilson's disease (WD). Notably, NAA/Cr demonstrated significant predictive capabilities (AUC=0.953, sensitivity=100%, specificity=88%), affirming its potential utility in assessing neurological involvement in WD, which is consistent with the findings emphasized by **Huang *et al.* [17]** regarding altered neurochemical ratios. In contrast, NAA/Cho displayed limited diagnostic utility (AUC=0.516, sensitivity=50%, specificity=63%) that is aligning with the observations of **Lafhal *et al.* [9]**, which has not extensively highlighted its role in predicting neurological symptoms in WD.

Our study investigated the diagnostic accuracy of the choline to creatine ratio (Cho/Cr) in predicting neurological manifestations in Wilson's disease (WD). Despite a non-statistically significant p-value of 0.156, Cho/Cr, with a cutoff point of 0.84, exhibited promising sensitivity (75%) and high specificity (94%). This observation aligns with the recognition by **Weinberg *et al.* [18]**, emphasizing the significance of Cho/Cr ratios in evaluating brain metabolism and neuronal health across various neurological conditions.

Our study contributed valuable insights into the diagnosis and assessment of neurological manifestations in pediatric WD, with neurochemical ratios, particularly NAA/Cr and Cho/Cr, emerging as promising predictors. These findings enhance the understanding of WD's neurological impact and potentially improving early diagnosis and patient care. Further research is warranted to advance the field's understanding of this complex disorder, ultimately benefiting WD patients in clinical practice.

CONCLUSION

Our study supports the clinical utility of N-acetyl aspartate (NAA) as a valuable biomarker for diagnosing Neurological manifestations in Wilson's disease (WD). The diagnostic accuracy of choline (Cho) and NAA/Cr ratios underscores their potential in assessing neurochemical alterations associated with WD's neurological spectrum. Despite the study's limitations, including sample size, these findings emphasized the importance of integrating neurochemical markers into a comprehensive diagnostic framework for a nuanced understanding of WD-related neurological changes.

RECOMMENDATIONS

1. **Clinical Integration:** Implementing N-acetyl aspartate (NAA) and choline (Cho) assessments in routine clinical evaluations for Wilson's disease (WD) patients to enhance early detection of Neurological manifestations.
2. **Comprehensive Approach:** Adopting a holistic approach by combining neurochemical markers, neuroimaging, and clinical assessments for a nuanced understanding of WD's diverse neurological changes.
3. **Neurochemical Ratios:** Exploration of the diagnostic potential of neurochemical ratios, particularly NAA/Cr, to refine the diagnostic toolkit for evaluating neurological symptoms in WD.

LIMITATIONS

1. **Sample size and heterogeneity:** Small sample size and patient heterogeneity may limit the generalizability of findings, emphasizing the need for larger and more diverse cohorts.
2. **Cross-sectional design:** The cross-sectional nature of the study provides a snapshot; longitudinal studies are crucial for understanding the progression of neurological manifestations in WD.
3. **Metabolic pathway interplay:** Further research is required to uncover the specific metabolic alterations influencing neurochemical ratios like NAA/Cho in WD patients.
4. **Diffusion MRI sensitivity:** While our diffusion MRI findings were inconclusive, future studies should explore advanced protocols and larger samples to capture nuanced neurological changes in WD.

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