

Wilson's Disease in Brain Magnetic Resonance Spectroscopy

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1. Introduction

Wilson's disease (WD) is an inherited disorder of copper metabolism first defined by Dr Samuel Alexander Kinnier Wilson in 1912 (Wilson, 1912). WD is caused by mutations to the gene coding for ATPase copper transporting beta polypeptide (ATP7B), which is located on 13 chromosome 13 and expressed in the liver (Bull et al., 1993; Loudianos et al., 1999; Yamaguchi et al., 1993; Frydman et al., 1985). The disease is inherited in an autosomal recessive manner.

WD is present worldwide in most populations, and particularly in those in which consanguineous marriage is common. The disease frequency is estimated to be between 1 in 5,000 and 1 in 30,000, and the heterozygous carriers (Hzc) frequency is approximately 1 in 90 (Figus et al., 1995).

In the ATP7B gene of WD a variety of mutations were detected. These defects include insertion, deletion, splice site and point mutations. In most ethnic groups, one or a small number of these ATP7B gene mutations are predominant, in addition to many other rare mutations. In Europeans and North Americans, two ATP7B point mutations: His1069Gln and Gly1267Arg account for 38% of mutations described in WD (Thomas et al., 1995). There is still no favorable correlation between phenotype and genotype, but frame shift deletions and nonsense mutations that cause a truncation of the translated protein product usually result in a severe form of the disease because of loss of the functional protein. The knowledge of the prevalence of mutations is helpful in achieving rapid mutational screening. Mutations in ATP7B cause a reduction in the conversion of apoceruloplasmin into ceruloplasmin, which, is therefore present at low levels in WD patients.

A failure to excrete copper into the biliary canaliculi leads to its toxic accumulation in the hepatocytes (Schilsky&Tavill, 2003). The copper excess may damage hepatic mitochondria and oxidative damage to hepatic cells and cause the spillage of copper into the blood, thereby overloading other organs such as the brain, kidney and red blood cells, initiating toxic damage (Schilsky&Tavill, 2003). In the early stages of WD diffuse cytoplasmic copper accumulation can be seen only by special immunohistochemical stains for copper detecting. The early accumulation of copper is associated with hepatic steatosis. The ultrastructural abnormalities range from enlargement and separation of the mitochondrial inner and outer membranes with widening of the intercrystal spaces, to increases in the density and

granularity of the matrix. During the disease progression periportal inflammation mononuclear cellular infiltration lobular necrosis and bridging fibrosis occur. The areas of the brain main affected in WD are the basal ganglia (lenticular nuclei), which macroscopically appear brown in color because of copper deposition (Scheinberg& Sternlieb, 1984). In the early stages of the WD, proliferation of large protoplasmic astrocytes such as Opalski cells and Alzheimer cells occurs. During the disease progression the degeneration occurs leading to necrosis, gliosis and cystic changes. The degeneration can be seen in the brainstem, thalamus, cerebellum and cerebral cortex. During the WD progression, copper deposits can lead to vacuolar degeneration in proximal renal tubular cells, appearance of the 'Kayser-Fleischer' (KF) ring in Descemet's membrane. Copper from the hepatic cells degradation can be released into the circulation and can damage red blood cells, thereby inducing hemolysis (Schilsky&Tavill, 2003).

The majority of patients with WD present or with hepatic or neuropsychiatric symptoms, and with either clinically asymptomatic or symptomatic liver involvement. The remaining patients, may present with symptoms attributable to the involvement of other organs, as acute non-immunological hemolytic anemia, osteoarthritis, arrhythmias, rheumatic-fever-like manifestation, renal function abnormalities, primary or secondary amenorrhea, repeated and unexplained spontaneous abortions. Patients with hepatic WD (hWD) usually present in late childhood or adolescence, and exhibit features of acute hepatitis, fulminant hepatic failure, or progressive chronic liver disease in the form of either chronic active hepatitis or cirrhosis of the macronodular type (Schilsky&Tavill, 2003; Hoogenraad, 1997). The mean age of onset of neurological WD (nWD) is the second to third decade (Hoogenraad, 1997). Most of patients present with extrapyramidal, cerebellar and cerebral-related symptoms. Tremor, gait and speech disturbances are the most common initial presentation symptoms. Patients may present also present with dystonia. About 30% of patients experience psychiatric disturbances (Hoogenraad, 1997). These disturbances can manifest as changes in school-related or work-related performance, attention deficit hyperactivity disorder, impulsivity, paranoid psychosis, obsessive behavior, depression, suicidal tendencies or bizarre behavior, and can occur early or late in the disease course.

The WD diagnosis and monitoring is based on history, physical, biochemical, liver biopsy and genetic mutations and imaging assessment. The KF ring is an important marker in nWD examination. The WD is biochemically characterized by low ceruloplasmin and total serum copper levels, increased 24-hour urinary copper excretion, and abnormally high hepatic copper content in the liver biopsy particularly in children performed. Another noninvasive method for assessing copper metabolism is incorporation of radioactive copper into the hepatocytes used with favorable results in Poland specially in presymptomatic gene mutation carriers, siblings of WD patients diagnosis (Członkowska et al. , 1973). The mutation screening to identify defects in the ATP7B gene can confirmation of WD diagnosis, but will not necessarily detect all disease producing mutations.

2. Neuroimaging

2.1 Magnetic resonance imaging

Neuroimaging plays an important role in the diagnosis and monitoring of WD patients. In brain CT scans hypodensities and atrophy of the bilateral basal ganglia, brainstem,

cerebellum and cerebral cortex can be seen (Hoogenraad, 1997). MRI is much more sensitive method for revealing abnormalities in WD (Hoogenraad, 1997; Sinha et al., 2006). On T1-weighted images, generalized brain atrophy is seen in about 75% of cases, and hypointensities in the basal ganglia. On T2-weighted images, hyperintensity in the basal ganglia, white matter, thalamus or brainstem can be seen (Figure 1). These abnormalities are caused by neuronal loss, gliosis, degeneration of fibers, and vacuolization associated with increased water content in the brain. Signal abnormalities vary according to the stage of the disease, and can be reversible with therapy in the early stages. In some cases, T2-weighted images show hypointensity in the basal ganglia (globus pallidus) region may be as a result of deposition of iron in exchange for copper after chelation (Figure 2).

In some WD patients with WD MRI shows a typical pallidal hyperintensity on T1-weighted images (Cordoba et al., 2003) (Figure 3). This abnormality appears to be secondary to the accumulation of manganese in basal ganglia because of portal-systemic shunting (Cordoba et al., 2003).

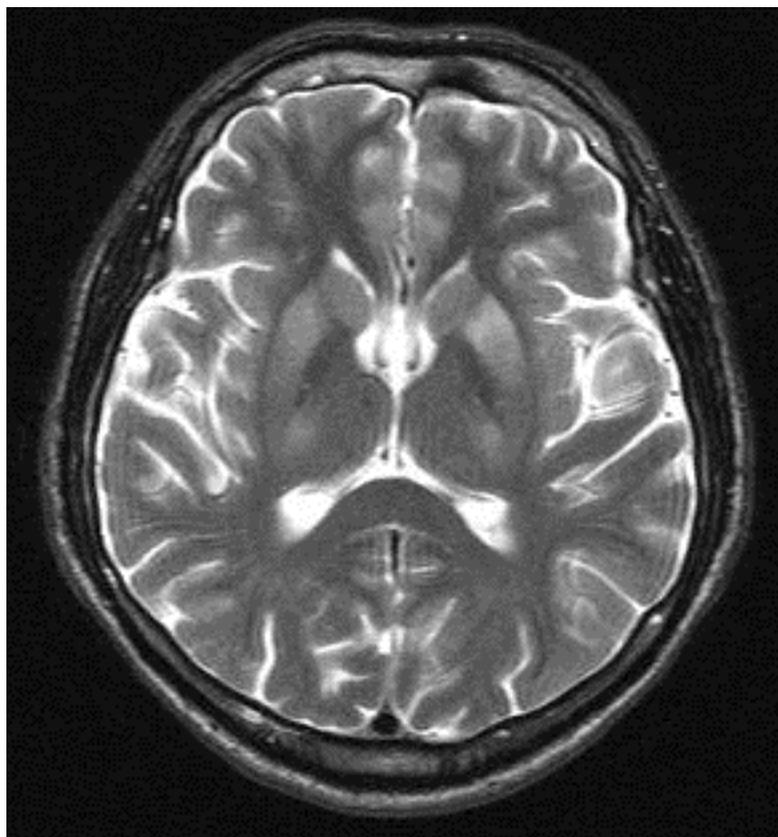


Fig. 1. Axial T2 - weighed MRI image of a 31- years - old men with WD, MRI was done before treatment. The picture shows bilateral hyperintensities in putamen, caudatum and thalami and degree of diffuse brain atrophy.

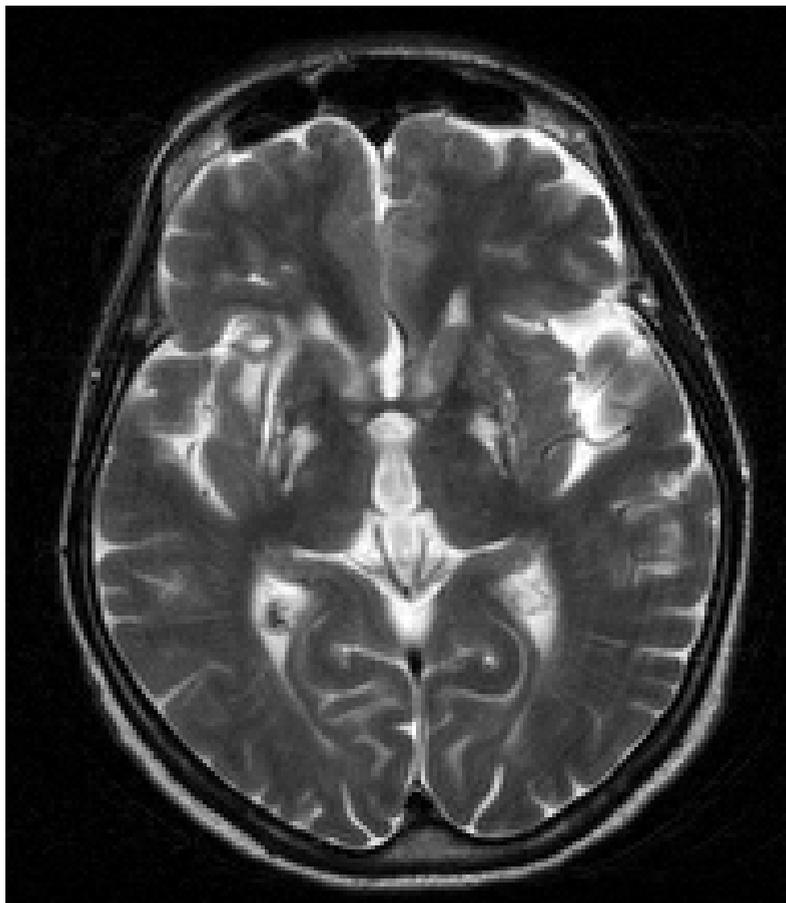


Fig. 2. Axial T2 - weighed MRI image of a 47- years - old women with WD treated for more than 10 years. The picture shows persistent hyperintensities in putamen, caudatum globus pallidus and degree of diffuse brain atrophy and hypointensity in the basal ganglia.

Proton Magnetic Resonance spectroscopy ($^1\text{H-MRS}$) is a practical research tool for elucidating the pathophysiology underlying certain diseases (Rudkin & Arnold, 1999). In patients with hepatic encephalopathy (HE), $^1\text{H-MRS}$ has been used to detect metabolic abnormalities in the brain with very high sensitivity (Ross et al., 1999). The biochemical alterations that were detected in HE included an increase in cerebral glutamine compounds (Glx) and a decrease in myoinositol (ml) and choline (Cho) metabolites (Cordoba et al., 2002). Other metabolites detectable *in vivo* by $^1\text{H-MRS}$ are N-acetylaspartate (NAA), considered to be a marker of neuronal health, and creatine (Cr), often used as an internal standard against which the resonance intensities of other metabolites are normalized. The role of NAA in nervous system can be related to: action as an organic osmolyte that counters the "anion deficit" in neurons, or a cotransport substrate for a proposed "molecular water pump" that removes metabolic water from neurons; NAA is a precursor for the enzyme-

mediated neuronal dipeptide N-acetylaspartylglutamate biosynthesis; NAA provides a source of acetate for myelin lipid synthesis and is involved in energy metabolism in neuronal mitochondria-reflect improvement of neuronal energetic (Moffett et al.,2007).

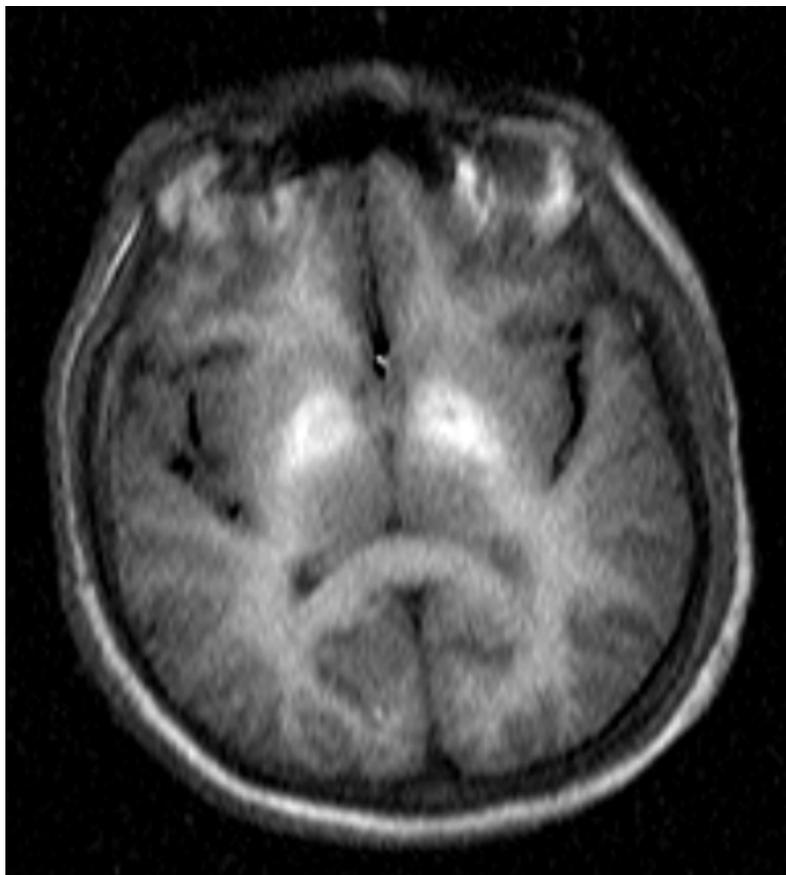


Fig. 3. Axial T1 - weighed MRI image of a 45- years - old WD patient with hepatic cirrhosis with subclinical hepatic encephalopathy.

^1H -MRS is a technique that could help distinguish between brain changes caused by HE and those related to copper toxicosis in WD. To elucidate the pathomechanism of the cerebral pathology of WD a study using ^1H -MRS in 37 newly diagnosed WD patients in the globus pallidus and thalamus examined bilaterally was performed (Tarnacka et al, 2009a). The calculations were performed for: ml, Cho, creatine (Cr), NAA, lipid (Lip), Glx. In all WD patients a significantly decreased ml/Cr and NAA/Cr ratio level and an increased Lip/Cr ratio in the pallidum was observed. Analysis revealed a significantly increased Glx/Cr and Lip/Cr ratio in the thalamus. In the pallidum of nWD patients, Cho/Cr and Glx/Cr and Lip/Cr ratios were higher than in control subjects, and the NAA/Cr was significantly lower (Table 1). In hWD patients, the ml/Cr and Cho/Cr and NAA/Cr ratio levels were lower

than in controls (Table 1). The Cho/Cr and Lip/Cr ratios were higher in the thalami of nWD patients, and Lip/Cr ratios were higher than controls' in patients only with hWD. On the Figure 4 the examples of ^1H -MRS spectra in nWD and hWD and presymptomatic patients compared with control subjects are provided.

Patients:	mI/Cr	Cho/Cr	Glx/Cr	NAA/Cr	Lip/Cr
nWD	$0,66 \pm 0,31$	$0,89 \pm 0,30^*$	$1,26 \pm 0,51^*$	$1,43 \pm 0,42^{\text{‡}}$	$0,94 \pm 0,46^*$
hWD	$0,50 \pm 0,27^{\text{‡}}$	$0,68 \pm 0,24^{\text{‡}}$	$1,38 \pm 0,39^*$	$1,36 \pm 0,39^{\text{‡}}$	$0,81 \pm 0,31^*$
presymptomatic	$0,35 \pm 0,10$	$0,66 \pm 0,09$	$1,37 \pm 0,24$	$1,59 \pm 0,14$	$0,65 \pm 0,08$
Control group	$0,72 \pm 0,15$	$0,78 \pm 0,15$	$1,11 \pm 0,25$	$1,61 \pm 0,26$	$0,47 \pm 0,20$

^1H -MRS study pallidum

Patients:	mI/Cr	Cho/Cr	Glx/Cr	NAA/Cr	Lip/Cr
nWD	$0,74 \pm 0,30$	$1,01 \pm 0,27^*$	$1,19 \pm 0,32$	$1,91 \pm 0,56$	$0,74 \pm 0,32^*$
hWD	$0,85 \pm 0,27$	$1,07 \pm 0,24$	$1,21 \pm 0,39$	$1,73 \pm 0,39$	$0,78 \pm 0,31^*$
presymptomatic	$0,76 \pm 0,21$	$0,91 \pm 0,23$	$1,13 \pm 0,20$	$1,69 \pm 0,32$	$0,50 \pm 0,22$
Control group	$0,82 \pm 0,20$	$0,83 \pm 0,14$	$1,11 \pm 0,21$	$1,68 \pm 0,24$	$0,50 \pm 0,20$

^1H -MRS.thalamus

*statistically higher than in control group

$^{\text{‡}}$ statistically lower than in control group

$^{\text{‡}}$ statistically lower compared with thalami

Table 1. The mean relative metabolic ratios in (left and right) pallidum and (right and left) thalamus in neurological (nWD) and hepatic (hWD) and presymptomatic subgroups.

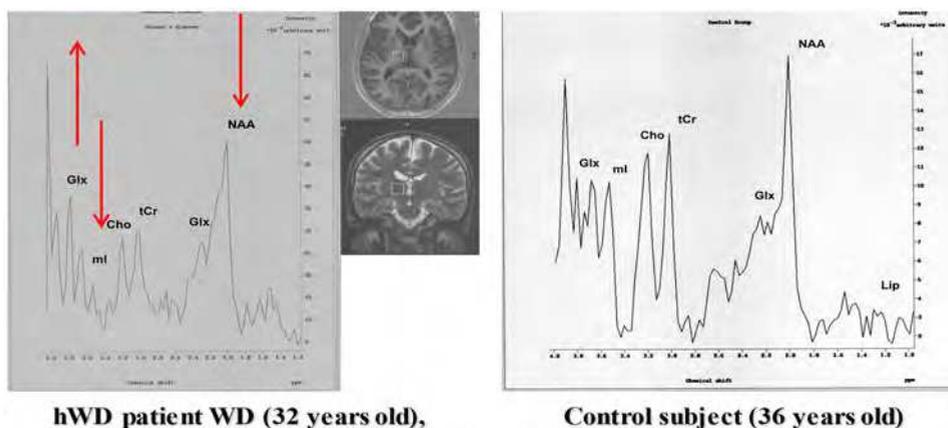


Fig. 4. a. ^1H -MRS images of patient with hepatic WD presentation compared with control subject. Proton spectrum shows expression of Glx; Cho and NAA were significantly reduced. The axial image shows the inset from where the spectrum was recorded.

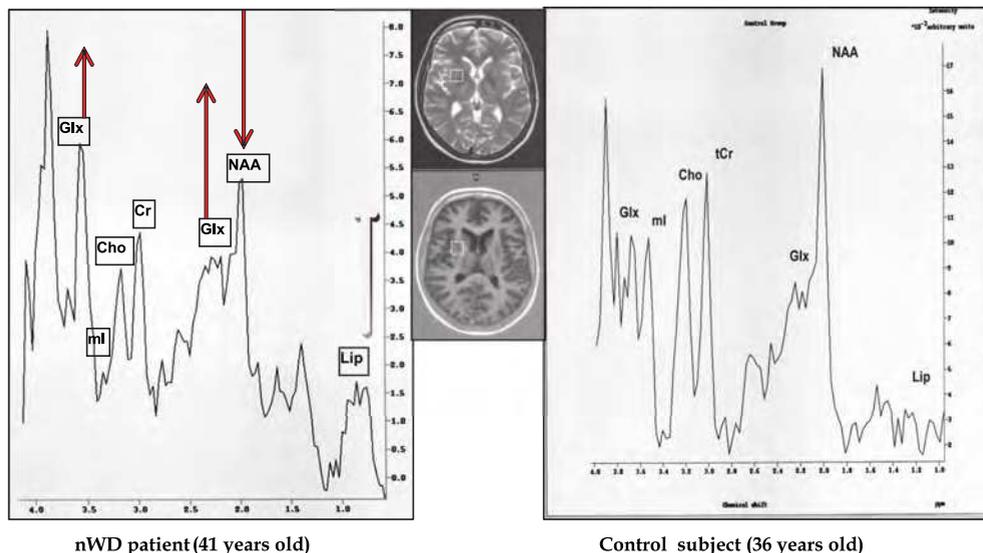


Fig. 4. b. ¹H-MRS images of patient with neurological WD presentation compared with control subject. Proton spectrum shows expression of Glx, Lip; NAA was significantly reduced. The axial image shows the inset from where the spectrum was recorded.

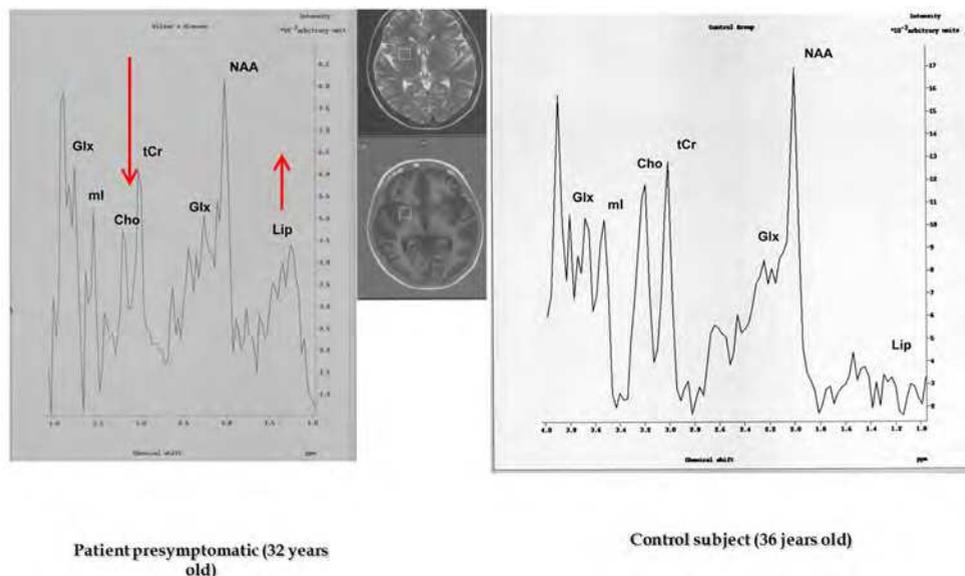


Fig. 4. c. ¹H-MRS images of presymptomatic WD patients compared with control subject. Proton spectrum shows expression of Lip; Cr was significantly reduced. The axial image shows the inset from where the spectrum was recorded.

In those study the thalamic changes compared with the basal ganglia were more sensitive to ongoing degenerative changes and portal-systemic encephalopathy.

Spectroscopic changes in presymptomatic patients were performed only on 4 cases, because of it was very difficult to find patients, who did not have any clinical and biochemical (liver failure) abnormalities. In those patients significantly lower levels of ml/Cr and Cho/Cr and increase of Glx/Cr ratios in basal ganglia. In thalami no changes were seen. Those findings can suggest that in presymptomatic patients in MRS early encephalopathic changes with decrease of ml can be noticed.

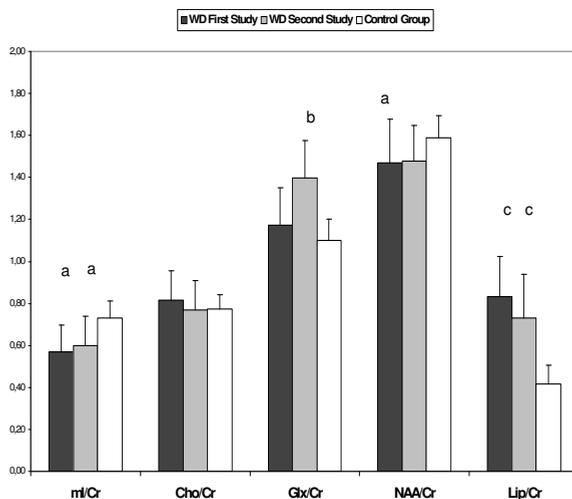
The decreased Cho/Cr and ml/Cr in the pallidum of hWD patients could be indicative of minimal HE. Mioinositol is one of the chief compatible organic osmolytes responsible for equilibrating an increased intracellular tonicity (Cho, is believed to be also an osmolyte) (Danielsen & Ross, 1999). The ml/Cr and Cho/Cr reduction was also reported by Kraft in one *de novo* WD patient with hepatic disease (Kraft E et al.,1999). In newly diagnosed WD patients a reduction of NAA/Cr in the pallidum of hWD patients was noted (Tarnacka et al, 2009). This could indicate that in these patients, ¹H-MRS detects a combination of early encephalopathic and neurodegenerative changes, if NAA can be considered a neuronal marker.

Newly diagnosed WD patients with neurological impairment showed an increased level of Cho/Cr and Lip/ Cr in the pallidum and thalamus; furthermore, Glx/Cr was increased and NAA/Cr was decreased in the pallidum. The Cho peak is considered a potential biomarker for the status of membrane phospholipid metabolism (Danielsen & Ross, 1999), so that an elevated Cho signal most likely reflects an increase in membrane turnover. In pathologies characterised by membrane breakdown, such as neurodegeneration, bound Cho moieties may be liberated into the free Cho pool (Danielsen & Ross, 1999). It is possible that the increase of Cho/Cr and Lip/Cr ratios in the pallidum and the thalami can reflect an increase in membrane turnover caused by free copper accumulation, or can be associated with gliosis because Cho is present in high concentrations in oligodendrocytes (Van der Hart et al., 2000). In nWD patients, the increased level of Glx/Cr in the pallidum, can be related to the shunting of ammonia to the brain and glutamine accumulation. The elevation of Glx, can be also related to neuronal energy impairment. Removal of glucose greatly accelerates glutamate transamination to aspartate in brain synaptosomes, suggesting that glutamate could be an energy source in brain under some circumstances (Erecinska et al, 1988). Aspartate transaminase is the enzyme that accomplishes the task of glutamate transamination. It consumes oxaloacetate and glutamate to produce aspartate and alpha-ketoglutarate, which can directly enter to the TCA cycle (Moffett et al, 2007). Most studies concerning the relationship between neurospectroscopic abnormalities and the neurological HE manifestation found an association between the presence of HE and Glx/Cr level (Kreis et al., 1992; Cordoba et al., 2001). In nWD patients no other metabolite changes specific for HE such as ml/Cr and Cho/Cr reduction was detected, so it is conceivable that because of chronic liver failure, in nWD patients the compensatory mechanism to counteract intracellular hypertonicity can take place, with no ml depletion, but with an increase of Glx. This finding, could indicate, that in newly diagnosed nWD patients an HE could exist. Patients with liver cirrhosis do present with parkinsonian signs including: tremor,

bradykinesia, dysarthria, hypomimia, or rigidity. Some authors have speculated that the neurological symptoms of WD can be caused by concomitant liver disease (Victor, 1999).

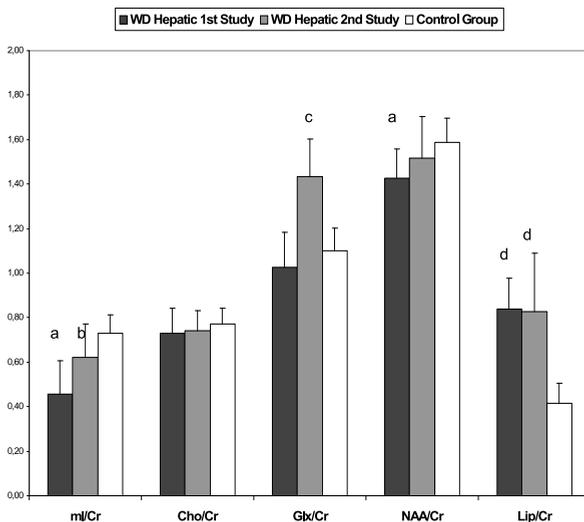
These MRS findings can prove that in newly diagnosed nWD patients in the brain a porto-systemic shunting changes, but also neurodegenerative pattern associated with Cho, Lip and NAA/Cr depletion nWD can coexist. Verma compared brain metabolite alternations in patients with acute liver failure, acute-on-chronic liver failure and chronic liver disease (Verma et al, 2008). He found that NAA/Cr ratio was significantly decreased in acute-on-chronic liver failure and chronic liver disease. This fact can prove that in chronic liver disease an mitochondrial dysfunction can be detected due to neurotoxic effect. It is also worthy to mentioned that in patients with hepatic and neurological impairment in WD a significant negative correlation in the pallidum between the clinical status and the NAA/Cr was detected (Tarnacka et al, 2009).

From the 37 patients newly diagnosed, we followed 17WD cases for more than one year period (Tarnacka et al, 2008). In the ^1H -MRS done during the follow-up of all WD patients, significantly lower levels of mI/Cr and higher levels of Lip/Cr compared to the control group were persistently noted (Figure 5a). In patients with hepatic signs with improvement, a statistically significant increase of mI/Cr and Glx/Cr in follow-up ^1H -MRS was observed (about one year post-treatment, Figure 5b). In patients with neurological improvement after treatment in the follow-up ^1H -MRS, a statistically significant increase of NAA/Cr was noted (Table 5c). During neurological deterioration in one case, a decrease of Glx/Cr and NAA/Cr was seen, in contrast to another neurologically impaired patient with liver failure exacerbation, where a decrease of mI/Cr and increase of Glx/Cr was observed (Tarnacka et al, 2009).



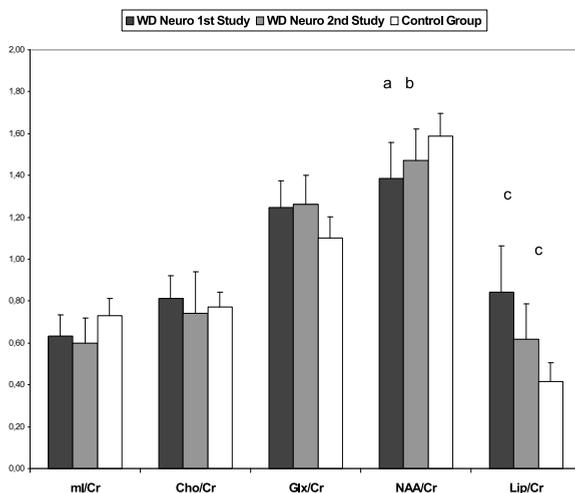
- a. significantly lower than in controls ($p < 0.005$),
 b. significantly higher than in controls ($p < 0.0001$) and compared with first study $p < 0.005$,
 c. significantly higher than in controls ($p < 0.005$),

(a)



- a. significantly lower than in controls, $p < 0.005$,
 b. significantly higher compared with first study $p < 0.04$,
 c. significantly higher than in controls, $p < 0.00006$ and compared with first study $p < 0.002$,
 d. significantly higher than in controls, $p < 0.005$

(b)



- a. significantly lower than in controls, $p < 0.05$,
 b. significantly higher compare with first study, $p < 0.01$
 c. significantly higher than in controls, $p < 0.02$

(c)

Fig. 5. The mean metabolic ratios in patients with improvement: a) all 15 WD patients b) hepatic subgroup ($n=9$), c) neurological subgroup ($n=6$), in the first and second ^1H -MRS test compared to the age-matched control subjects. Statistical analysis in text.

The alternations of NAA/Cr ratio in neurologically impaired patients and ml/Cr and Glx/Cr in patients with liver failure could be a sensitive marker of the clinical recovery and deterioration in those WD patients.

A decrease in relative NAA concentrations has been observed in pathological processes known to involve neuronal loss (Rudkin & Arnold, 1999). NAA is synthesized by neuronal mitochondria, which are sensitive to injury (Bates et al., 1996). A reversible decrease of NAA could well be caused by mitochondrial toxin 3-nitropropionate (Dauntry et al., 2000). Mitochondrial proteins seem to be the target of copper toxicity, because a decrease in the

	ml/Cr	Cho/Cr	Glx/Cr	NAA/Cr	Lip/Cr
All	0.66±0.30	0.89±0.21 ^a	1.35±0.20 ^b	1.44±0.35	0.93±0.48 ^c
CS	0.83±0.14	0.75±0.16	1.09±0.31	1.61±0.22	0.45±0.18
hWD	0.63±0.33	0.79±0.38	1.33±0.47	1.47±0.38	0.99±0.57 ^d
CS	0.66±0.14	0.76±0.16	1.09±0.31	1.58±0.22	0.43±0.18
nWD	0.72±0.21	0.96±0.36 ^e	1.36±0.44 ^f	1.41±0.42	0.89±0.48 ^g
CS	0.95±0.41	0.75±0.12	1.09±0.23	1.63±0.28	0.46±0.22

a. significantly higher than in controls ($p < 0.0002$)

b. significantly higher than in controls ($p < 0.0005$)

c. significantly higher than in controls ($p < 0.00002$)

d. significantly higher than in controls ($p < 0.000000$)

e. significantly higher than in controls ($p < 0.00002$)

f. significantly higher than in controls ($p < 0.004$)

g. significantly higher than in controls ($p < 0.0003$)

(a)

	ml/Cr	Cho/Cr	Glx/Cr	NAA/Cr	Lip/Cr
All	0.68±0.30	0.79±0.36	1.10±0.37	1.36±0.26 ^a	0.94±0.31 ^b
CS	0.71±0.18	0.76±0.16	1.13±0.21	1.65±0.21	0.47±0.16
hWD	0.64±0.22	0.58±0.21 ^c	1.31±0.18	1.45±0.50	0.87±0.50 ^d
CS	0.78±0.28	0.93±0.24	1.20±0.27	1.56±0.31	0.50±0.22
nWD	0.75±0.34	1.03±0.30 ^e	1.18±0.32	1.30±0.25 ^f	0.86±0.29 ^g
CS	0.71±0.17	0.65±0.16	1.03±0.22	1.60±0.29	0.47±0.20

a. significantly lower than in controls ($p < 0.01$),

b. significantly higher than in controls ($p < 0.003$)

c. significantly lower than in controls ($p < 0.001$)

d. significantly higher than in controls ($p < 0.0003$)

e. significantly higher than in controls ($p < 0.0003$)

f. significantly lower than in controls ($p < 0.002$)

g. significantly higher than in controls ($p < 0.003$)

(b)

Table 2. The mean metabolites ratios compared with control subjects (CS) in all WD patients, in hWD and nWD subgroups in: a) patients with improvement of clinical status, b) patients with no improvement of clinical status.

levels of the subunits of some of the complexes of the respiratory chain occurs (complex I being the most affected) (Aricello et al., 2005). It is possible that in WD, functional changes in neurons affecting oxidative metabolism may result in the reversible changes in relative concentration of NAA are reported in those study here. These findings suggest, that the early diagnosis and treatment of WD patients can reduce neurons metabolic disturbances and avoid their degeneration process. It is a very useful information which can prove that in nWD patients with improvement of clinical signs in the brain reversible functional changes of neurons could be detected.

In WD patients treated for long time in nWD and hWD subgroups with improvement or no improvement of clinical status the MRS findings in Table 2 are provided (Tarnacka et al., 2010). In those study we investigated 4 hWD patients, with no improvement and 8 with marked improvement; and 8 nWD patients with marked improvement and 7 with no improvement of clinical status. In hWD patients with improvement the MRS did not show any important pathological changes and in the nWD with improvement significantly higher Cho/Cr, Glx/Cr and Lip/Cr ratios levels compared with controls were noted (Table 2a). In hWD patients with no improvement the lower Cho/Cr and in nWD significantly lower NAA/Cr and higher Cho/Cr and Lip/Cr ratios were detected (Table 2b).

In all WD patients who showed improvement after treatment, MRS revealed a higher level of Cho/Cr, Glx/Cr and Lip/Cr ratios. In our previous study, we demonstrated a decrease in NAA/Cr ratio in all symptomatic patients, which increased after one year of treatment (Tarnacka et al., 2008). In patients treated for a longer time with improvement of clinical status no NAA/Cr decrease was seen; this could suggest that in WD patients the NAA/Cr depletion is reversible during the first few years of treatment and that treatment with z-s or d-p can protect them from further WD progression. The Cho/Cr and Glx/Cr elevation may mirror the glial proliferation that can be detected in patients even after long-term treatment (Horoupian et al., 1988). In this study, the persistence of NAA/Cr was related to a lack of improvement in neurological status (Tarnacka et al., 2010), what is in concordance with earlier reports in the literature showing a lower level of NAA in the striatum in neurologically impaired patients who received treatment (Page et al., 2004; Lucato et al., 2005). The lower level of NAA/Cr in the pallidum in these patients may suggest that, in patients with neurological involvement, persistent neuronal dysfunction may occur as a result of copper and/or iron deposition. NWD patients with no improvement initially had lower ceruloplasmin levels compared with those in whom neurological recovery was noted. It is possible that in these patients the iron metabolism could also be disturbed because of ceruloplasmin deficiency, which can be enhanced during chelation treatment (Medici et al., 2007).

2.1.1 MRS in heterozygous WD gene carriers

Wilson's disease a genetic disease due to mutation of both alleles of "Wilson's disease" gene ATP7b. The number of heterozygotes - having only one faulty copy of the gene - is high, around 1-2% of the human population (Johnson, 2001). Heterozygote carriers should not have symptoms of hepatic, neurological dysfunctions, but in other recessive disorders such as phenylketonuria, some deviations in brain function in the literature were reported (Vogel, 1984). Because ^1H -MRS is able to detect brain abnormalities that are invisible in clinical assessment and MRI a study using this method to investigate the probability of brain

changes in Hzc was performed (Tarnacka et.al, 2009b). We observed statistically significant higher ratios of Glx/Cr and Lip/Cr in ¹H-MRS in Hzc in the pallidum (Table 3). Glx is a glial marker; the increase of Glx/Cr could correlate with the glial proliferation as a result of the protective function of the astrocytes to copper and iron accumulation. The significantly higher level of Lip/Cr may reflect the liberation of lipids from membranes during their breakdown as a reaction to copper and iron overload. Our results may instead suggest that WD Hz carriers accumulate free copper or iron or both in basal ganglia and thalami. Hzc in our study were much older than WD cases are and in an animal model of WD copper and iron levels were found to be augmented during aging in striatum and substantia nigra (Kim et al., 2001). It is possible that during aging in Hz there is an increase of copper and iron accumulation because of ATP7b and ceruloplasmin impairment. There is accumulating evidence that ceruloplasmin, a copper protein with ferroxidase activity, plays an important role in iron metabolism and is also the principal plasma copper-binding protein (Harris et al., 1998). In our Hz the ceruloplasmin level was lower in only four cases, but ceruloplasmin levels in the blood are influenced by acute-phase reactions. The copper accumulation in the brain can also be caused by disruption in its excretion. In animal models, heterozygous mice have a reduced ability to excrete copper, which may indicate that half of the normal liver ATP7b copper transporter activity is insufficient to deal with a large amount of copper intake (Cheach et al., 2007). Like iron, copper is a heavy metal that can induce the Fenton reaction via production of free radicals and copper and iron accumulation increased with aging might have deleterious of liver and brain via oxidative stress.

	mI/Cr	Cho/Cr	Glx/Cr	NAA/Cr	Lip/Cr
¹ H-MRS study					
Gp					
Hz:	0.73±0,19	0.81±0,13	1.38±0,30*	1.5±0,28	0.96±0,40**
Control subjects	0.82 ± 0,82	0.78 ± 0,16	1.11 ± 0,26	1.62 ± 0,27	0.48 ± 0,20
¹ H-MRS study					
Th					
Hz	0.90 ± 0,27	0.95 ± 0,23	1.44± 0,40‡	1.93± 0,35	1.08± 0,40†
Control subjects	0.82± 0,21	0.84 ± 0,15	1.12 ± 0,21	1.69 ± 0,25	0.51 ± 0,20

*significantly higher than in controls, p<0.00006

** significantly higher than in controls, p<0.00002

‡ significantly higher than in controls, p<0.0006

† significantly higher than in controls, p<0.000001

Table 3. The mean metabolites ratios in heterozygotes (Hz) and control subjects from left and right globus pallidus (Gp) and right and left thalamus (Th) with standard deviations.

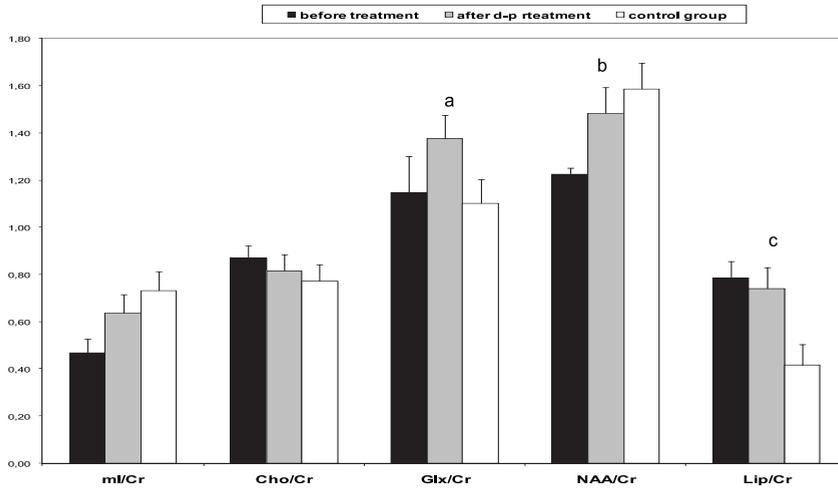
2.1.2 MRS versus Wilson's disease treatment

The treatment of WD patients may take many forms, from drug therapy (chelating agents as d-penicillamine (d-p) or trientine, zinc therapy, tetrathiomolibdate) to liver transplantation

(LT). The aim of medical treatment for WD is to remove the toxic deposit of copper from the body to produce a negative copper balance, and to prevent its reaccumulation. Successful therapy is measured in terms of a restoration of normal levels of free serum copper and its excretion in the urine. In 9 patients treated for more than one year with d-p in author's preliminary study, statistically significant increase of Glx/Cr and NAA/Cr ratios was noticed in contrary to 8 patients treated with zinc sulphate (z-s) were decrease of ml/Cr and NAA/Cr was detected (Figure 6a,b). D-penicillamine seems to be more effective (increase of NAA/Cr) during one year treatment, compared with z-s, but those study was performed on a quite small sample of patients. Those findings can also confirm Brewers observations, that zinc compounds are slow acting drugs and the decrease of ml/Cr can reflect the metabolic deterioration during zinc therapy (Brewer, 2005).

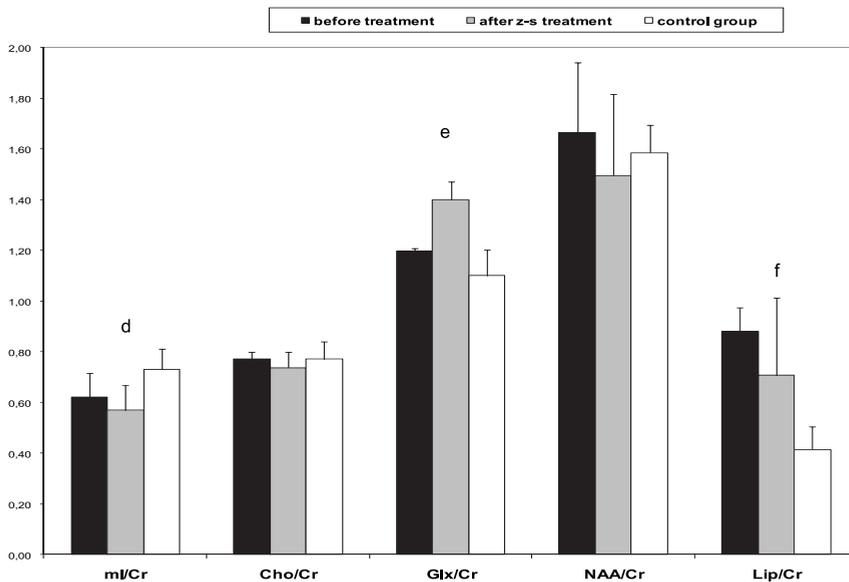
Current therapeutic strategies are often effective in the treatment of WD and provide a negative copper balance, however the course of WD in some treated patients may be unpredictable (Członkowska et al., 2005). The effectiveness of chelation or zinc therapy should therefore be monitored. So far, the only useful tests of monitoring the treatment efficiency are laboratory and imaging methods. Biochemical tests used in monitoring WD treatment include plasma liver tests, caeruloplasmin, copper and zinc in serum, and urinary copper and zinc excretion tests. These methods may sometimes be not sufficiently in particular at the beginning of the treatment when most of treatment side-effects occur as iatrogenic worsening during d-p therapy. In this stage we postulated that ^1H -MRS can be used in early stages of treatment monitoring when the alternations of NAA, ml and Glx can mirror therapeutic response or deterioration (Tarnacka et al, 2008). We think that ^1H -MRS can also be helpful in differential diagnosis of clinical deterioration in patients with WD.

In patients with progressive liver failure or acute liver failure from fulminant hepatitis with or without intravenous hemolysis, orthotropic hepatic transplantation is an efficient treatment (Hoogenraad, 1997). Hepatic transplantation is also indicated in the absence of liver failure in patients with neurological WD in whom chelation therapy has proved ineffective, and significant improvements in neurological features have been reported (Polson et al., 1987). We presented a study of spectroscopic changes in globus pallidus in 3 patients with WD undergoing LT. The first case was a patient with neurological and hepatic impairment who was undergoing LT because of liver cirrhosis exacerbation. The second and third patients were subjects only with hepatic signs and the LT was performed because of liver failure. In the patient with neurological signs the MRS was performed 4 months after LT because there was no neurological improvement, and again one year after that. In the first MRS in those patient there was an increase in Glx/Cr, NAA/Cr and Lip/Cr ratios levels compared with the controls (Figure 7a). After one year's observation time an improvement of clinical status was observed and no changes of metabolites ratios were seen (Figure 7a). Before LT in the patient with liver cirrhosis ml/Cr ratio level was very low, in both patients with liver failure a decreased level of Glx/Cr, NAA/Cr and increased Lip/Cr ratios levels compared with controls were found (Figure 7b,c). After LT in those patients an increase of ml, Glx/Cr and NAA/Cr was seen (Figure 7b,c). Those data provide that after LT in those WD patients a renormalization of brain metabolites changes detected in ^1H -MRS could be seen.



- a. statistically higher than in controls ($p < 0,0003$),
 b. statistically higher than before treatment ($p < 0,01$),
 c. statistically higher than in controls ($p < 0,00004$),

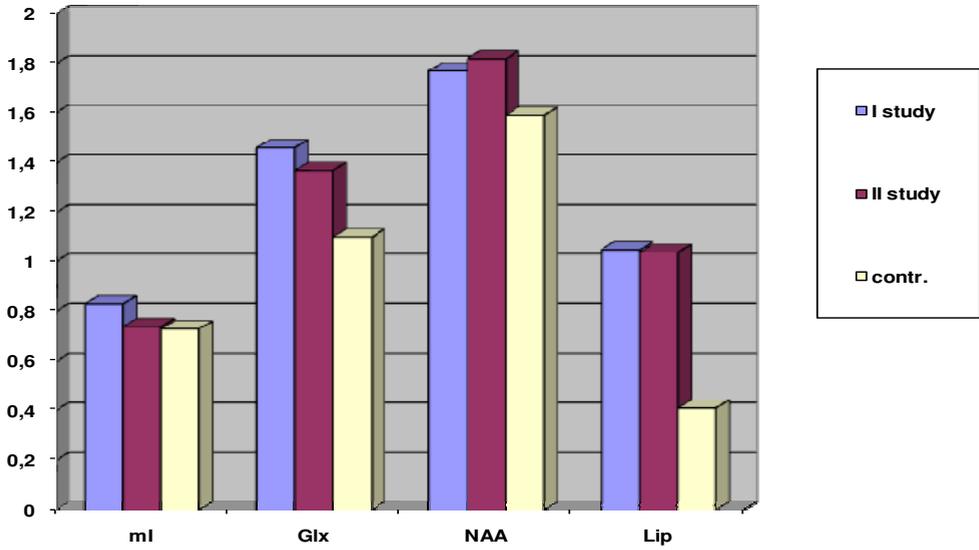
a)



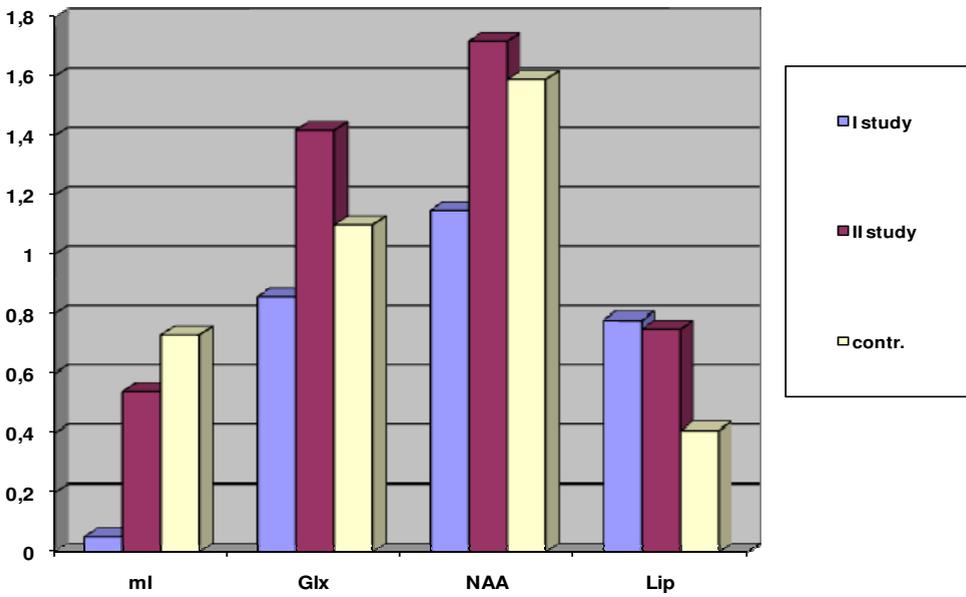
- a. significantly higher than in controls ($p < 0,003$),
 b. significantly higher than in controls ($p < 0,0002$),
 c. significantly higher than in controls ($p < 0,001$).

b)

Fig. 6. The mean metabolites ratios before treatment with d-p (a) and z-s (b), after minimum one year of treatment and in control subjects.



a)



b)

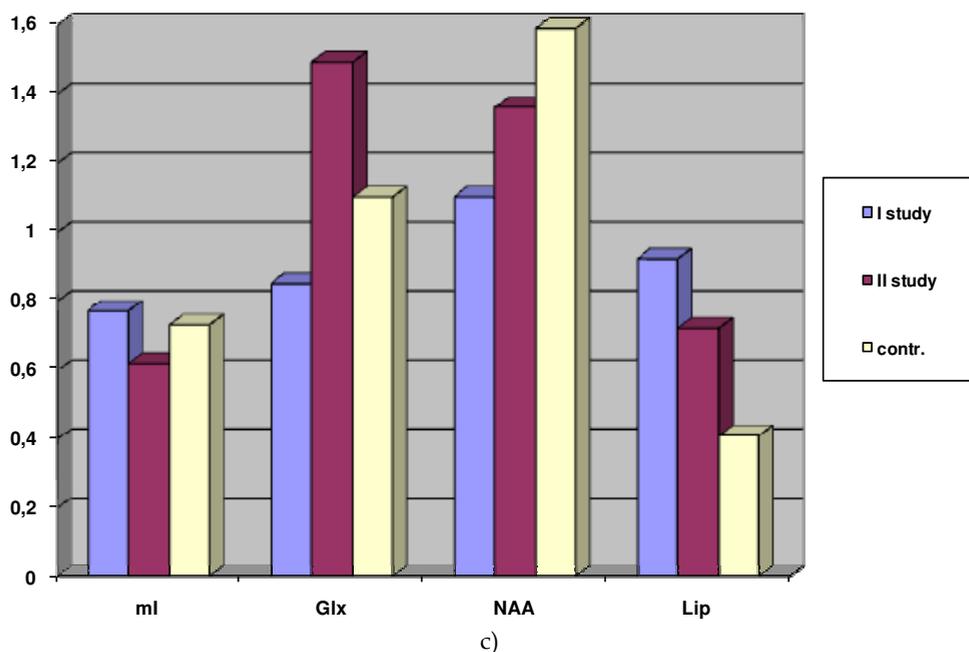


Fig. 7. The mean metabolites ratios in globus pallidus in 3 patients undergoing liver transplantation, in first patient a) second- b) third- c), explanation in the text.

3. Conclusion

In conclusion, the results of the present studies appear to prove that in WD the astrocytic abnormalities occur primarily with dysfunction of the astrocytic-neuronal interactions. This process leads to metabolic derangement in neurons. During the disease progression and increasing copper deposition in the brain the compensation reactions as metallothionein synthesis become inefficient and the degenerative changes of astrocytes prevail over their detoxicative possibilities. The copper liberates from damaged astrocytes, and can also damage the neurons directly. Free copper can induce the neuronal mitochondria dysfunction causing the impaired NAA synthesis, but on this stage the destructive process can be reversible, because the increase of NAA/Cr can be observed in most treated patients. This is a very important conclusion, because it shows that the first year of treatment are pivotal in the therapeutic process. In majority of patients in this stage the central nervous system damage can be reversible. These findings emphasize the need for early diagnosis and treatment of WD. Effective antioxidant therapy introduced in early stages of WD might have modifying effect of metabolic disturbances in neurons. The neuronal dysfunction lasting longer can cause non reversible degenerative process like in patients treated for long time with no neurological improvement. Irreversible astrocytic dysfunction caused by copper can be related with disturbed ceruloplasmin synthesis causing iron overload and lead to lack of neurological improvement in some WD patients. The ^1H -MRS technique can be useful in treatment monitoring in patients with WD in early stages (until one year). MRS seems to be more effective method in comparison with MRI

in monitoring effectiveness of treatment during first year. MRS could be helpful in differential diagnosis of the clinical deterioration in treated WD patients. The alternations of NAA/Cr ratio in neurologically impaired patients and ml/Cr and Glx/Cr in patients with liver failure could be a sensitive markers of the clinical recovery and deterioration in those WD patients. MR spectroscopy could be a useful complementary tool in other monitoring treatment methods in WD patients.

4. Acknowledgment

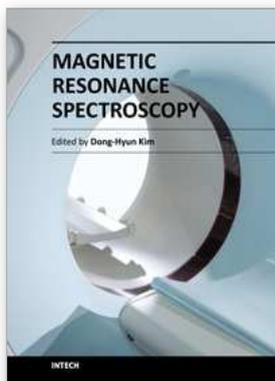
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Magnetic Resonance Spectroscopy (MRS) is a unique tool to probe the biochemistry in vivo providing metabolic information non-invasively. Applications using MRS has been found over a broad spectrum in investigating the underlying structures of compounds as well as in determining disease states. In this book, topics of MRS both relevant to the clinic and also those that are beyond the clinical arena are covered. The book consists of two sections. The first section is entitled 'MRS inside the clinic' and is focused on clinical applications of MRS while the second section is entitled 'MRS beyond the clinic' and discusses applications of MRS in other academic fields. Our hope is that through this book, readers can understand the broad applications that NMR and MRS can offer and also that there are enough references to guide the readers for further study in this important topic.

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