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BRIEF ARTICLE

Wilson disease: Histopathological correlations with treatment on follow-up liver biopsies

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Abstract

AIM: To investigate the progression of hepatic histopathology in serial liver biopsies from Wilson disease (WD) patients.

METHODS: We report a group of 12 WD patients treated with zinc and/or penicillamine who underwent multiple follow-up liver biopsies. Demographic, clinical and laboratory data were gathered and all patients underwent an initial biopsy and at least one repeat biopsy.

RESULTS: Time to repeat biopsy ranged from 2 to 12 years. Six patients (non-progressors) showed stable hepatic histology or improvement. In one case, we observed improvement of fibrosis from stage 2 to 0. Six patients (progressors) had worsening of fibrosis. There was no significant correlation between the histological findings and serum aminotransferases or copper metabolism parameters. The hepatic copper concentration reached normal levels in only two patients: one from the non-progressors and one from the progressors group. The estimated rate of progression of hepatic fibrosis in the entire group was 0 units per year in the time frame between the first and the second liver biopsy (4 years), and 0.25 between the second and the third (3 years). In the progressors group, the rate of progression of liver fibrosis was estimated at 0.11 fibrosis units per year between the first and second biopsy and, 0.6 fibrosis units between the second and third biopsy.

CONCLUSION: The inability of clinical tools to detect fibrosis progression in WD suggests that a liver biopsy with hepatic copper quantification every 3 years should be considered.

Key words: Wilson disease; Copper; Liver biopsy; Histopathology

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INTRODUCTION

Wilson disease (WD) is an inherited, autosomal recessive disorder of copper accumulation that affects about one individual per 30 000 population[1]. It is due to the dysfunction of a copper-transporting P-type ATPase that has a crucial role in copper excretion into the bile. The gene that encodes this P-type ATPase, ATP7B, is located on chromosome 13q14.3, and numerous gene mutations can impair the protein's function[2,3], which leads to copper accumulation mainly in the liver, but also in the brain, cornea, and kidney. The most frequent clinical presentation of WD is liver involvement[4]. Hepatic manifestations may vary from hepatomegaly and fatty liver, to acute hepatitis, with high serum aminotransferases, liver failure, jaundice, and cirrhosis. The earliest morphological features of WD are represented by micro- and macrovesicular hepatic steatosis, glycogenated nuclei in the periportal hepatocytes, and focal hepatocellular necrosis. With the progression of parenchymal damage and inflammation, fibrosis, and subsequently cirrhosis, invariably develop. Cirrhosis can have either a micronodular or a mixed macro-micronodular pattern, and it is rarely complicated by hepatocellular carcinoma or cholangiocarcinoma[5]. Regarding the timing of the disease progression, cirrhosis is often diagnosed by the second decade, but there are some individuals who do not develop cirrhosis even after the fourth decade of life[6,7]. The ultrastructural analysis is characterized typically by mitochondrial abnormalities, including variability in size and shape, increased density of the matrix material, and numerous inclusions of lipid and fine granular material, which may be copper[8]. With adequate treatment, these changes may not occur. WD is a treatable disorder and early diagnosis is essential: the goal of therapy is to reduce copper accumulation by enhancing its urinary excretion (with chelating agents) and by decreasing its intestinal absorption (with zinc salts)[9-11]. As a result of the rarity of the disease and the fact that the liver biopsy is not performed routinely during the follow-up of WD, unless clinically indicated, the progression and timing of the liver pathology and its correlation with different anti-copper treatments or with aminotransferase levels are poorly characterized. Previous studies have shown the possibility of improvement of the steatosis and inflammation grade[12], and the fibrosis stage[13,14] during long-term follow-up. However, studies on serial liver biopsies, as well as studies on the correlation between hepatic histology and clinical parameters, are lacking.

The overall objective of this study was to describe the evolution of liver histology in WD patients during penicillamine (PCA) and zinc treatment, to define the rate of progression of the liver damage, and to correlate the clinical and biochemical parameters of liver injury with hepatic copper concentration.

MATERIALS AND METHODS

We included 12 patients with WD from the Division of Gastroenterology and Hepatology, Padua University Hospital (Italy), who were followed from 1981 to 2006 and who underwent serial liver biopsies. The mean follow-up was 5 ± 3 years (range: 1-12 years). Patients with history of alcohol abuse, positive serology for hepatitis B and C, and features of the metabolic syndrome were excluded. The study was conducted according to the principles of the Declaration of Helsinki, and all patients gave informed consent before undergoing liver biopsy. WD was diagnosed when 24-h urine copper excretion was > 100 μg/24 h, hepatic copper concentration was > 250 μg/g dry weight, and serum ceruloplasmin was < 20 mg/dL. All patients were treated with either PCA or zinc sulfate following the initial biopsy. Liver biopsy samples were obtained by the percutaneous route, using the Menghini method. Liver copper concentrations in dried liver tissue were measured by flame atomic absorption spectrophotometry. Demographic, clinical, and laboratory data were gathered, and all patients underwent an initial and at least one repeat biopsy. Selected laboratory values were recorded at the time of initial and repeat biopsies: serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), 24-h urinary copper excretion, and hepatic copper concentration. Slides of formalin-fixed, paraffin-embedded tissue were stained with hematoxylin and eosin (HE), and with the histochemical stains Perls for hemosiderin and Van Gieson for fibrosis. Two experienced pathologists who scored the biopsies were blinded to all clinical and biochemical information, and to the order of execution of the biopsies. The biopsies were semi-quantitatively graded on three main parameters (inflammation, steatosis, and fibrosis). The grading of portal and lobular inflammation was 0, none; 1, mild; 2, moderate; and 3, severe. The grading of steatosis was 0, none; 1, up to 25%; 2, 25%-50%; 3, 50%-75%; and 4, 75%-100%. The staging of fibrosis was 0, none; 1, expansion of portal fibrous tissue; 2, early bridging, no nodules; 3, bridging fibrosis, early nodule formation; and 4, cirrhosis. Iron deposits were graded by a standard method, from 0 to 4, where grade 1 represents minimal iron deposition and grade 4 represents iron deposition throughout all zones[15]. Patients were separated into two groups. We considered as progressors the patients who presented worsening of at least one unit of fibrosis. Inflammation and steatosis were described separately. Non-progressors presented stable or improved fibrosis scores. The rate of the fibrosis progression was calculated as the result of the mean difference in fibrosis scores divided by the mean interval in years between the first and second liver biopsies.

Statistical analysis

Data are expressed as mean ± SD or as proportions. The goodness-of-fit test was used to determine whether the distributions of continuous variables were normal prior to analysis[16]. Measurements were log-transformed as necessary to improve the normality of residuals and homoscedasticity (or homogeneity of variance) of errors before analysis. Paired *t* test was used to compare the underlying mean differences in response between follow-up and baseline. Separate analyses were performed for each variable and each follow-up time. Analysis of variance (ANOVA) was performed to assess: (1) whether there was a significant mean difference in each response between two independent samples (i.e. treatment groups); and (2) whether there was a significant mean difference between progressors and non-progressors, after controlling the potential effects of other variables. Pearson (Spearman) correlation coefficients were estimated to assess the magnitude and direction of a linear association between two given continuous (ranked) variables. Individual trajectories of serum measurement changes in response level over the follow-up time were estimated from linear random-effect models. Each response level was entered as the dependent variable and treatment, follow-up time, and treatment × follow-up time interaction were entered as the independent variables. To account for inter-subject heterogeneity in the change of response level, intercept and time were modeled as random effects. A two-side *P* value of 0.05 was considered significant. All statistical analyses were performed using SAS, Version 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Clinical features

Table 1 summarizes the clinical features for progressors *vs* non-progressors. The mean age of the 12 patients at diagnosis was 17.3 years (range: 6-35 years), and three (25%) were female. Ten patients presented with different degrees of hepatic symptoms and two with mixed hepatic and neurological disease, mainly characterized by rigidity, tremors, and dystonia. At baseline, before anti-copper treatment, 24-h urinary copper concentration was 994 ± 1293 μg/24 h, and mean hepatic copper concentration was 491 ± 260 μg/g dry liver. Five patients were started on PCA after the diagnosis, and the remaining seven on zinc salts. Most patients remained on the same drug during follow-up. One patient began zinc treatment that was later changed to PCA, and the therapy switch was indicated by the lack of improvement of liver enzymes. At diagnosis, the mean ALT was 84.1 ± 50.9 U/L, and AST was 62.8 ± 50.5 U/L. During follow-up, we observed a significant improvement of aminotransferase levels, with mean ALT 37.3 ± 20.6 U/L and AST 35.3 ± 34.8 U/L (P = 0.01 and 0.03, compared to baseline) at the time of the second liver biopsy and mean ALT 38.3 ± 17 U/L and AST 27.9 ± 9.9 U/L at the time of the third liver biopsy. There was no significant change in hepatic copper concentration over time (Figure 1), and there was no significant difference in terms of laboratory data improvement between progressors and non-progressors (Table 1).

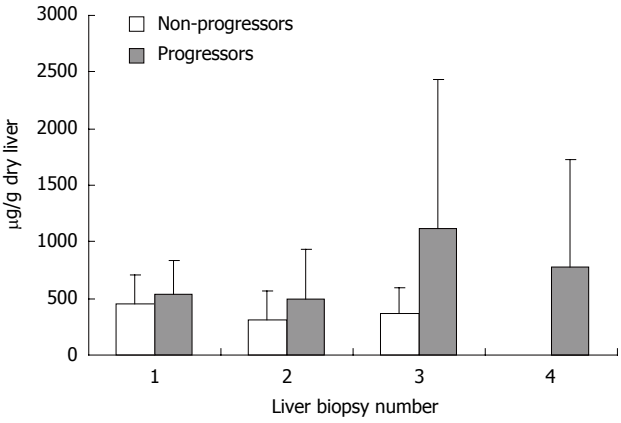

Figure 1 Evolution of the hepatic copper concentration in patients who presented with progression and without progression of the total histological score. Progressors had a higher hepatic copper concentration at all time points, but the difference between the two groups was not significant.

Hepatic histology

All patients underwent at least two liver biopsies. The average number of portal tracts was 14.9 ± 7.5 (range: 4-34). The mean interval between the first and the second liver biopsy was 4 years (range: 2-12 years) (12 patients). The mean interval between the second and third biopsy was 3 years (range: 2-4 years) (four patients). Two patients underwent a fourth liver biopsy after 2 and 3 years, respectively. None of the patients was on anti-copper treatment at the time of the first liver biopsy. The follow-up liver biopsies were indicated by an increase in the aminotransferase levels, or change of therapy, or were performed to monitor the potential disease progression. At baseline, five patients had grade 0 steatosis, three had grade 1, three had grade 2, and one presented with grade 3. Regarding the stage of fibrosis, seven had stage 0, three had stage 1, and two had stage 2. There were no cirrhotic patients. Three patients (two on zinc and one on PCA) showed overall improvement of the histological severity (patients 2, 3 and 6), with decreased steatosis (Figure 2A and B), and in one case, significant improvement of fibrosis, from stage 2 to 0 over 6 years of follow-up (Figure 2C and D). Three patients (one on zinc and two on PCA) showed no significant change in histology, although initial biopsies in this group showed mild lesions (patients 1, 4 and 5; Table 2). The patients with mixed neurological and hepatic phenotype were both included in the non-progressors group (patients 1 and 2). The six patients who manifested histological progression (patients 7-12) demonstrated worsening of inflammation and/or fibrosis (Figure 2E and F). Of these six patients, four had been started on zinc and two on PCA, and one was switched from zinc to PCA. One patient (#10) who showed an overall progression underwent the second liver biopsy 12 years after the first one, and over this time, the grade of steatosis and inflammation and the stage of fibrosis increased (Table 2).

Table 1 Baseline characteristics of patients who showed progression of the histologic score (progressors) *vs* those who showed overall improvement or no progression in histology (non-progressors)

	Progressors	Non-progressors
Age at diagnosis (mean ± SD) (yr)	18.0 ± 8.8	16.6 ± 9.8
Phenotype	All hepatic phenotype	4 with hepatic phenotype; 2 with mixed hepatic and neurological phenotype
AST U/L (baseline) (normal range 15-43)	60.0 ± 56.8 (range 18-44) (6)	64.6 ± 51.3 (range 15-129) (6)
AST U/L (time of 2nd biopsy)	45.1 ± 48.2 (range 20-143) (6)	25.5 ± 9.7 (range 13-39) (6)
AST U/L (time of 3rd biopsy)	27.3 ± 10.2 (range 13-44) (6)	28.7 ± 10.8 (range 15-41) (3)
AST U/L (time of 4th biopsy)	24 and 28 (2)	
ALT U/L (baseline) (normal range 6-43)	83.5 ± 40.2 (range 45-137) (6)	84.5 ± 60.7 (range 15-164) (6)
ALT U/L (time of 2nd biopsy)	48.5 ± 21.8 (range 24-80) (6)	26.1 ± 12.3 (range 10-46) (6)
ALT U/L (time of 3rd biopsy)	42.0 ± 17.2 (22 and 68) (2)	32.7 ± 17.2 (range 8-48) (3)
ALT U/L (time of 4th biopsy)	47.0 ± 7.0 (42 and 52) (2)	
24 h urinary Cu μg/24 h (baseline)	679.7 ± 504.0 (6)	1245.0 ± 1649.0 (6)
Hepatic Cu mg/g dry liver (baseline)	534.4 ± 298.7 (6)	455.0 ± 245.3 (6)
Type of treatment	2 = penicillamine; 4 = zinc One patient switched to penicillamine during follow up	3 = penicillamine; 3 = zinc No change of treatment during follow up

The numbers in brackets represent the number of patients included in the analysis.

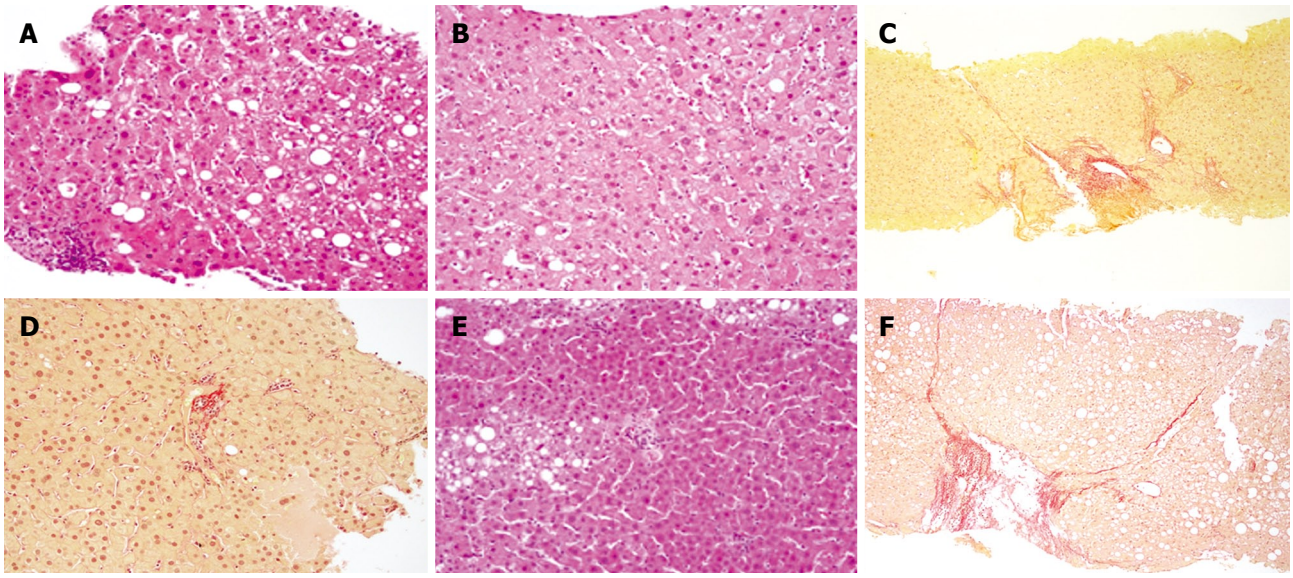

Figure 2 Biopsy of patients 3 and 9. A: Patient 3, initial biopsy, showing steatosis and focal inflammation (HE stain, 200 ×); B: Patient 3, third biopsy, 6 years after diagnosis, showing only rare steatosis and no inflammation (HE stain, 200 ×); C: Patient 3, initial biopsy, with bridging fibrosis (HE stain, 100 ×); D: Patient 3, second biopsy, without portal fibrosis (HE stain, 100 ×). Patient 3 was treated with zinc; E: Patient 9, initial biopsy, showing focal steatosis (HE stain, 200 ×); F: Patient 9, fourth biopsy, 8 years after diagnosis, showing bridging fibrosis and mild inflammation (Van Gieson stain, 100 ×). Patient 9 was treated with zinc.

Hepatic copper concentration was variable in the two groups, and did not correlate with histological findings. Progressors showed a mean hepatic copper concentration higher than non-progressors at all time points, but the result was not significant due to the small sample and high variability of the hepatic copper content (Figure 1). In our study, the hepatic copper concentration reached normal levels (< 50 μg/g dry weight liver) in only two patients: one from the non-progressors group and one from the progressors group. The patients who did not show any change in histology also had consistent decreases in hepatic copper concentration, although levels were still elevated above normal. Iron staining was positive at baseline in two progressors and in one non-progressor. In one non-progressor the iron staining was positive after 4 years of anti-copper treatment (Table 2). There was no correlation between iron staining results and the severity of inflammation and fibrosis.

The histological progression did not correlate with subsequent aminotransferase levels or with the type of therapy. The estimated rate of progression of hepatic fibrosis (as result of the mean difference in fibrosis scores divided by the mean interval in years between the first and second liver biopsies) in the entire group was 0 units per year in the time frame between the first and the second liver biopsy, and 0.25 between the second and the third. However, among progressors, the rate of progression of fibrosis was estimated at 0.23 fibrosis units per year between the first and the second biopsy, and 0.6 units between the second and the third.

Table 2 Histopathology score of serial liver biopsies in WD patients who showed improved or stable hepatic fibrosis

Patient	Biopsy interval (yr)	Biopsy data			Iron staining	Treatment type
		Inflammation	Steatosis	Fibrosis		
Group NP						
1[1]		0	0	0	None	-
	2	0	0	0	None	Zinc
2[1]		0	2	0	None	-
	5	0	1	0	None	Zinc
3		2	1	2	None	-
	3	0	0	0	None	Zinc
	3	0	0	0	None	Zinc
4		0	1	1	3+, diffuse	-
	2	0	1	0	3+, diffuse	PCA
5		0	0	0	None	-
	4	0	0	0	None	PCA
6		0	2	0	Not done	-
	2	1	1	0	None	PCA
	4	1	1	0	2+, focal	PCA
Group P						
7		0	2	0	None	-
	3	1	1	2	None	Zinc
8		1	1	0	Not done	-
	2	1	1	0	None	Zinc
	3	1	1	2	None	Zinc
	2	2	1	2	None	Zinc
9		0	3	1	1+, Kuppfer	-
	3	0	3	1	None	Zinc
	2	1	3	2	None	Zinc
	3	1	3	2	None	Zinc
10		1	0	2	None	Zinc
	12	2	1	3	None	PCA
11		0	0	0	2+	-
	5	1	0	2	None	PCA
12		0	0	1	None	-
	5	1	1	2	None	PCA

Grade of inflammation and steatosis are also described. [1]Indicates the two patients who presented with mixed hepatic and neurological phenotype. NP: Non-progressors; P: Progressors; Zinc: Zinc sulfate; PCA: Penicillamine.

DISCUSSION

While our study confirms that, in WD, the clinical laboratory parameters do not correlate with the progression of hepatic histopathology, our newest finding is the rate of progression of fibrosis of 0 fibrosis units per year over a mean follow-up of 4 years after the diagnosis, and of 0.25 over 3 years between the second and third liver biopsy. We also observed improvement of the stage of fibrosis in two patients. Although some patients have been followed for several years, the overall amount of fibrosis in our study was low, with no patients demonstrating cirrhosis, even after long-term follow-up. However, our study covered a maximum of 12 years of follow-up, which might not be a sufficient time to observe the development of advanced-stage fibrosis in WD. A large study from Germany on 163 patients, 78 of whom underwent liver biopsy, showed variable hepatic involvement, with 37% patients presenting with cirrhosis, 36% with unspecified stage of fibrosis, and 54% with steatosis. Similar to our data, the hepatic copper concentration was highly variable, with a range from 95 to 3776 μg/g dry weight[17].

Our finding that anti-copper treatments, zinc and PCA, were equally distributed between progressors and non-progressors is in agreement with previous studies that have demonstrated various responses to different type of treatments, including PCA, trientine, and zinc. There are six main previous studies on follow-up liver biopsies including a total of 42 WD patients (Table 3). The effect of PCA in the long-term progression of liver damage in WD has been described in three small groups of patients: four pediatric patients showed improvement or stable hepatic fibrosis after 2-7 years of treatment[18], and seven adult patients showed marked improvement or disappearance of steatosis and improvement of mitochondrial morphological abnormalities after 3-5 years of PCA[19]. Shiono *et al*[20] have described an improvement in chronic active hepatitis in one patient after 6 years of PCA treatment, while in two patients with cirrhosis, there was no significant change in histopathology after 3-8.5 years of follow-up. Marcellini *et al*[12] have described a pediatric population of 22 subjects that underwent a follow-up liver biopsy 10 years after diagnosis, and all subjects were treated with zinc sulfate. The

Table 3 Review of the case series describing the evolution of hepatic histology in WD

Grand *et al*[18], 1975						Treatment: PCA	
Interbiopsy interval 2-7 yr		Hepatic copper (μg/g dry weight)		AST/ALT		Histopathology	
	Age at diagnosis (yr)	Before	After	Before	After	Before	After
1	24	NA	400	NA	NA	Inflammation 3+; connective tissue 13%; fatty vacuolization 0.5%	Inflammation 1+; connective tissue 7%; fatty vacuolization 0.5%
2	18	NA	80	NA	NA	Inflammation 2+; connective tissue NA; fatty vacuolization NA	Inflammation 2+; connective tissue 3%; fatty vacuolization 2%
3	11	1360	757	NA	NA	Inflammation 3+; connective tissue 17.6%; fatty vacuolization 5%	Inflammation 0/1+; connective tissue 7.6%; fatty vacuolization 3%
4	13.5	1112	90	NA	NA	Inflammation 4+; connective tissue 16%; fatty vacuolization 13%	Inflammation 0; connective tissue 14%; fatty vacuolization 8%
Sternlieb *et al*[19], 1976						Treatment: PCA	
Interbiopsy interval 3-5 yr		Hepatic copper (μg/g dry weight)		AST/ALT		Histopathology	
	Age at diagnosis (yr)	Before	After	Before	After	Before	After
1	15	821	109	52/59	39/20	Mild fibrosis; steatosis	Mild fibrosis; marked diminution of steatosis
2	9	1004	945	76/136	20/44	Steatosis	Marked diminution of steatosis
3	12	866	737	180/190	24/26	NA	NA
4	15	1123	239	22/94	16/22	Severe steatosis	Resolution of severe steatosis
5	10	832	453	57/88	53/48	Inflammation; mild fibrosis; steatosis	Resolution of inflammation; mild fibrosis; diminution of steatosis
6	12	1177	1050	65/85	45/43	Inflammation; severe fibrosis and steatosis	Resolution of inflammation; diminution of severe steatosis and fibrosis
7	14	NA	NA	75/82	50/35	Inflammation; steatosis; cirrhosis	Diminution of steatosis and inflammation; persistence of cirrhosis
Shiono *et al*[20], 2001						Treatment: PCA	
Interbiopsy interval 3-8.5 yr		Hepatic copper (mg/g dry weight)		ALT (IU/L)		Histopathology (only stage of fibrosis)	
	Age at diagnosis (yr)	Before	After	Before	After	Before	After
1	16	990	319	121	65	Cirrhosis with chronic active hepatitis	Cirrhosis
2	17	1025	200	241	109	Chronic active hepatitis	Chronic inactive hepatitis
3	19	524	190	17	25	Cirrhosis	Cirrhosis
4	23	540	129	19	18	Cirrhosis	Cirrhosis
Marcellini *et al*[12], 2005						Treatment: zinc sulfate	
Interbiopsy interval 10 yr		Hepatic copper (median of 22 pts) (mg/g dry weight)		AST/ALT (mean of 22 pts)		Histopathology summary of 22 pts	
		Before	After	Before	After	Before	After
Mean age at diagnosis (yr) 6.1 ± 2.5		873 (670-982)	690 (600-890)	110/94	21.7/23.7	Inflammation grade 1 in 81% of pts, grade 0 in 19%; steatosis grade 1 in 50%, grade 2 in 22.7%, grade 3-4 in 27.3%; fibrosis stage 1 in 54%, stage 3 in 46%	Resolution of inflammation in all pts; steatosis grade 1 in 90%, grade 2 in 10%; fibrosis stage 1 in 81%, stage 3 in 19%
Askari *et al*[13], 2003						Treatment: trientine + zinc, followed by long-term zinc	
Interbiopsy interval 4.4-10 yr		Hepatic copper		Child Pugh score (AST/ALT not available)		Histopathology (only stage of fibrosis)	
		Before	After	Before	After	Before	After
Mean age at diagnosis (yr) 25.4 ± 3.9		NA	NA	9-13	5	Cirrhosis Cirrhosis Cirrhosis	Fibrosis stage 2-3 Fibrosis stage 3-4 Fibrosis stage 1
Linn *et al*[14], 2009						Treatment: zinc	
Interbiopsy interval 3-7 yr		Hepatic copper (mg/g dry weight)		ALT (U/L)		Histopathology	
	Age at diagnosis (yr)	Before	After	Before	After	Before	After
1	21	NA	NA	59	57	Mild fibrosis	Normal
2	13	1100	270	31	19	Normal	Cirrhosis

authors observed an improvement in all parameters of histological damage (inflammation, steatosis, and fibrosis) and an overall decrease in hepatic copper concentration; however, the level remained higher than normal in all patients[12]. Askari *et al*[13] have shown various degrees of improvement of fibrosis in three WD patients with cirrhosis, who were treated first with zinc and trientine and later only with zinc as maintenance treatment. One patient showed persistent stage 3-4 fibrosis; a second patient showed stage 2-3 and only one showed stage 1. Linn *et al*[14] have described 17 patients who were followed for a median of 14 years and treated with zinc. In two cases, a second liver biopsy was performed after the baseline, which showed resolution of the initial mild fibrosis in one case and development of cirrhosis in the other. Although the data are heterogeneous and the progression of histopathological features is described following different criteria, it seems that there was an improvement in histology in most of the described patients, while we observed improvement or no progression only in 50% of cases. The explanation of our findings may be that we performed the liver biopsies when clinically indicated by the failure to respond to anti-copper agents, which potentially selected worse cases.

In previous work on WD, there have been variable changes in serum aminotransferases upon initiation of either PCA or zinc therapy, as well as persistently high levels resistant to either PCA or zinc therapy[10,21]. In none of these studies was there a significant correlation between aminotransferase level and histological progression, which confirms the observation made by us and others that, in WD, an elevation of aminotransferase level is common and does not correspond to clinical worsening[22]. The discovery of a higher hepatic copper concentration in progressors, as compared to non-progressors, is certainly not surprising and it may underline the importance of measuring hepatic copper during follow-up. Only one post-treatment specimen became positive for iron staining, and there was no correlation with fibrosis progression, which prevented us making any comparison with the results of Shiono *et al*[20], which showed hepatic iron accumulation after long-term anti-copper treatment. Our study was limited by the relatively small number of patients; however, this was still one of the largest studies conducted on serial biopsies of this rare condition. The limited statistical power did not allow us to find significant correlations or predictive factors of histological progression, but we were able to derive important observations that might contribute to the long-term management of WD, considering that the timing and the indication for follow-up liver biopsy in WD has not been established yet. Our patients were recruited in the hepatology setting and were selected for this study according to the availability of serial liver biopsies. Nevertheless these patients' varied histopathology appears representative of the WD hepatic presentation. Our study focused on the role of liver biopsy in WD follow-up. However, liver biopsy, even as a fundamental diagnostic tool in WD and in chronic liver diseases, is limited by sampling error, which can affect both histological evaluation and hepatic copper concentration, which is known to be significantly variable over time and among regenerative nodules in WD cirrhosis[23,24]. Despite these limitations, our data are particularly valuable because of the rarity of WD and the infrequency of serial liver biopsies in this disease. Our observation of the inability of clinical tools to detect the progression of fibrosis despite treatment suggests that a liver biopsy with hepatic copper quantification every 3 years should be considered.

COMMENTS

Background

The earliest morphological features of Wilson disease (WD) are represented by micro- and macrovesicular hepatic steatosis, glycogenated nuclei in the periportal hepatocytes, and focal hepatocellular necrosis. With the progression of parenchymal damage and inflammation, fibrosis, and subsequently, cirrhosis invariably develop. As a result of the rarity of WD and the fact that liver biopsy is not performed routinely during follow-up of WD, unless clinically indicated, the progression and timing of the liver pathology is characterized poorly. Previous studies have shown the possibility of improvement during long-term follow-up of the steatosis and inflammation grade, and of the fibrosis stage. Studies on serial liver biopsies, as well as studies on the correlation between hepatic histology and clinical parameters are lacking.

Research frontiers

The research hotspots are: (1) what is the rate of hepatic fibrosis progression in WD; and (2) when is the best time to perform follow-up liver biopsies in WD patients?

Innovations and breakthroughs

The results indicate that the estimated rate of progression of hepatic fibrosis (as result of the mean difference in fibrosis scores divided by the mean interval in years between the first and second liver biopsies) in the entire WD group was 0 units per year between the first and second liver biopsy (4 years), and 0.25 between the second and third (3 years). However, among progressors the rate of progression of liver fibrosis was estimated as 0.23 and 0.6 fibrosis units per year between the first and second biopsy and between the second and third, respectively.

Applications

The results suggest that liver biopsy with hepatic copper quantification every 3 years should be considered.

Peer review

This paper investigate the progression of hepatic histopathology in serial liver biopsies from WD patients. The manuscript is well written, and it can be published in current form.

REFERENCES

1 **Frydman M**. Genetic aspects of Wilson's disease. *J Gastroenterol Hepatol* 1990; **5**: 483-490

2 **Bull PC**, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat Genet* 1993; **5**: 327-337

3 **Tanzi RE**, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, Romano DM, Parano E, Pavone L, Brzustowicz LM. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993; **5**: 344-350

4 **Medici V**, Rossaro L, Sturniolo GC. Wilson disease--a practical approach to diagnosis, treatment and follow-up. *Dig Liver Dis* 2007; **39**: 601-609

5 **Guan R**, Oon CJ, Wong PK, Foong WC, Wee A. Primary hepatocellular carcinoma associated with Wilson's disease in a young woman. *Postgrad Med J* 1985; **61**: 357-359

6 **Ludwig J**, Moyer TP, Rakela J. The liver biopsy diagnosis of Wilson's disease. Methods in pathology. *Am J Clin Pathol*

1994; **102**: 443-446
7 **Stromeyer FW**, Ishak KG. Histology of the liver in Wilson's disease: a study of 34 cases. *Am J Clin Pathol* 1980; **73**: 12-24
8 **Sternlieb I**. Mitochondrial and fatty changes in hepatocytes of patients with Wilson's disease. *Gastroenterology* 1968; **55**: 354-367
9 **Roberts EA**, Schilsky ML. Diagnosis and treatment of Wilson disease: an update. *Hepatology* 2008; **47**: 2089-2111
10 **Medici V**, Trevisan CP, D'Incà R, Barollo M, Zancan L, Fagiuoli S, Martines D, Irato P, Sturniolo GC. Diagnosis and management of Wilson's disease: results of a single center experience. *J Clin Gastroenterol* 2006; **40**: 936-941
11 **Sturniolo GC**, Mestriner C, Irato P, Albergoni V, Longo G, D'Incà R. Zinc therapy increases duodenal concentrations of metallothionein and iron in Wilson's disease patients. *Am J Gastroenterol* 1999; **94**: 334-338
12 **Marcellini M**, Di Ciommo V, Callea F, Devito R, Comparcola D, Sartorelli MR, Carelli G, Nobili V. Treatment of Wilson's disease with zinc from the time of diagnosis in pediatric patients: a single-hospital, 10-year follow-up study. *J Lab Clin Med* 2005; **145**: 139-143
13 **Askari FK**, Greenson J, Dick RD, Johnson VD, Brewer GJ. Treatment of Wilson's disease with zinc. XVIII. Initial treatment of the hepatic decompensation presentation with trientine and zinc. *J Lab Clin Med* 2003; **142**: 385-390
14 **Linn FH**, Houwen RH, van Hattum J, van der Kleij S, van Erpecum KJ. Long-term exclusive zinc monotherapy in symptomatic Wilson disease: experience in 17 patients. *Hepatology* 2009; **50**: 1442-1452
15 **Searle J**, Kerr JFR, Halliday JW, Powell LW. Iron storage disease. In: MacSween RNM, Anthony PP, Scheuer PJ, Portmann BC, Burt AD, editors. Pathology of the liver. 3rd ed. Churchill Livingstone: Edinburgh, 1994: 219–241
16 **D'agostino RB**, Stephens M. Goodness-of-Fit Techniques, 2nd ed. New York: Marcel Dekker, Inc, 1986
17 **Merle U**, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. *Gut* 2007; **56**: 115-120
18 **Grand RJ**, Vawter GF. Juvenile Wilson disease: histologic and functional studies during penicillamine therapy. *J Pediatr* 1975; **87**: 1161-1170
19 **Sternlieb I**, Feldmann G. Effects of anticopper therapy on hepatocellular mitochondria in patients with Wilson's disease: an ultrastructural and stereological study. *Gastroenterology* 1976; **71**: 457-461
20 **Shiono Y**, Wakusawa S, Hayashi H, Takikawa T, Yano M, Okada T, Mabuchi H, Kono S, Miyajima H. Iron accumulation in the liver of male patients with Wilson's disease. *Am J Gastroenterol* 2001; **96**: 3147-3151
21 **Iorio R**, D'Ambrosi M, Marcellini M, Barbera C, Maggiore G, Zancan L, Giacchino R, Vajro P, Marazzi MG, Francavilla R, Michielutti F, Resti M, Frediani T, Pastore M, Mazzarella G, Fusco G, Cirillo F, Vegnente A. Serum transaminases in children with Wilson's disease. *J Pediatr Gastroenterol Nutr* 2004; **39**: 331-336
22 **Iorio R**, D'Ambrosi M, Marcellini M, Barbera C, Maggiore G, Zancan L, Giacchino R, Vajro P, Marazzi MG, Francavilla R, Michielutti F, Resti M, Frediani T, Pastore M, Vegnente A. Persistence of elevated aminotransferases in Wilson's disease despite adequate therapy. *Hepatology* 2004; **39**: 1173-1174
23 **Goldfischer S**, Sternlieb I. Changes in the distribution of hepatic copper in relation to the progression of Wilson's disease (hepatolenticular degeneration). *Am J Pathol* 1968; **53**: 883-901
24 **Faa G**, Nurchi V, Demelia L, Ambu R, Parodo G, Congiu T, Sciot R, Van Eyken P, Silvagni R, Crisponi G. Uneven hepatic copper distribution in Wilson's disease. *J Hepatol* 1995; **22**: 303-308

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