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【 CASE REPORT 】

Importance of a Liver Biopsy in the Management of Wilson Disease

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Abstract:
A 37-year-old Wilson disease patient treated with D-penicillamine visited our hospital for the evaluation of his liver function. Laboratory data showed a low serum copper level and ceruloplasmin. The ratio of urinary copper to urinary creatinine in a spot urinary analysis after 4 days' cessation of D-penicillamine was under 0.1. We concluded that the copper chelation was excessive and changed D-penicillamine to zinc acetate. However, his liver function test results did not normalize. We performed a liver biopsy and discovered a high copper content. The liver dysfunction was improved after resuming chelating therapy. Accurate measurement of the hepatic copper content via a biopsy is important for the adequate management of this disease.

Key words: Wilson disease, liver biopsy, hepatic copper content

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Introduction

Wilson disease is an autosomal recessive genetic disorder characterized by an impaired excretion of copper into the bile and the accumulation of copper in various organs. Wilson disease affects 1 in every 30,000 individuals with a carrier frequency of 1 in every 80 (1-3). If Wilson disease is diagnosed at an early stage, patients are treatable, and the long-term prognosis is favorable (3). Although clinical practice guidelines for Wilson disease have been proposed by the American Association for the Study of Liver Diseases, the European Association for the Study of the Liver (1, 2, 4) and by Japanese medical authorities (in Japanese), the diagnosis and treatment remain difficult in some cases.

We herein report a patient with Wilson disease who was treated with D-penicillamine or zinc acetate. Although laboratory data indicated a good therapeutic effect, the liver specimen obtained by a biopsy revealed a high copper content. The measurement of the intrahepatic copper content by a liver biopsy is important for the assessment of treatment.

Case Report

A 37-year-old man, who had been taking D-penicillamine at a dose of 600 mg/day for Wilson disease, visited our hospital for the evaluation of his liver function in 2012. He had been diagnosed with Wilson disease at 20 years of age by detailed examinations, including a liver biopsy, detection of Kayser-Fleischer rings and a genetic analysis.

Laboratory data showed low serum copper and ceruloplasmin levels at the first visit to our hospital. The ratio of urinary copper to urinary creatinine in a spot urinary analysis after 4 days' cessation of D-penicillamine was under 0.1, and the serum ferritin level was high (Table 1). Therefore, we concluded that his copper chelating therapy was excessive and replaced D-penicillamine with zinc acetate. However, his liver function test results did not normalize.

In 2015, we performed a liver biopsy to evaluate the exact hepatic copper content and assessed the 24-hour urinary

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Table 1. Laboratory Data at the Initial Visit.

Hematology		CK	271 U/L	Immunology	
WBC	3,400 /μL	BUN	13 mg/dL	IgG	1,128 mg/dL
RBC	490×10^4 /μL	Cr	0.68 mg/dL	ANA	<×40
Hb	14.1 g/dL	Na	137 mmol/L		
MCV	85.1 μm^3	K	4.1 mmol/L	Coagulation	
PLT	16.3×10^4 /μL	Cl	107 mmol/L	PT%	92.4 %
		Fe	58 μg/dL	APTT	32.4 sec
Blood chemistry		Ferritin	460 ng/mL		
TP	6.8 g/dL	Cu	10 μg/dL	Viral marker	
Alb	4.2 g/dL	Ceruloplasmin	<2 mg/dL	HBs Ag	(-)
T-Bil	0.5 mg/dL			HBc Ab	(-)
AST	38 U/L	Serology		HCV Ab	(-)
ALT	32 U/L	CRP	0.05 mg/dL		
ALP	286 U/L			Urinalysis (spot urine)	
γ-GTP	55 U/L			Cu	11 μg/dL
LDH	186 U/L			Cr	367.9 mg/dL

Table 2. Laboratory Data on Admission.

Hematology		AST	45 U/L	Serology	
WBC	3,500 /μL	ALT	50 U/L	CRP	0.01 mg/dL
RBC	479×10^4 /μL	ALP	275 U/L		
Hb	13.9 g/dL	γ-GTP	58 U/L	Coagulation	
MCV	83.1 μm^3	LDH	219 U/L	PT%	89.5 %
PLT	17.0×10^4 /μL	CK	269 U/L		
		BUN	11 mg/dL	Tumor marker	
Blood chemistry		Cr	0.80 mg/dL	PIVKA-II	23.0 mAU/mL
TP	7.0 g/dL	Na	137 mmol/L	AFP	3.0 ng/mL
Alb	4.4 g/dL	K	4.0 mmol/L		
T-Bil	0.7 mg/dL	Cl	103 mmol/L	Urinalysis (24 hour urine)	
		Ceruloplasmin	<2 mg/dL	Cu	49.2 μg/day

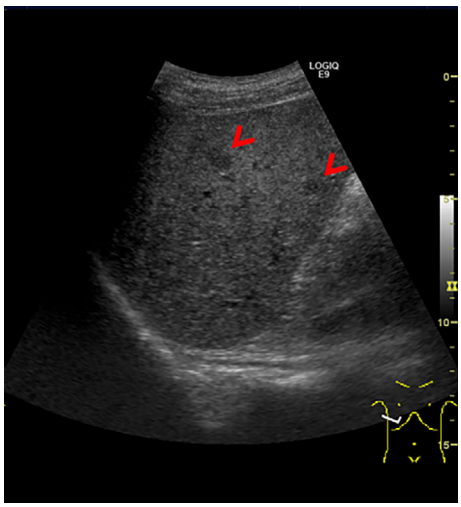

Figure 1. An abdominal ultrasound examination showed mild irregularity of the liver surface, mild interior coarseness, brightness, and multiple hypoechoic lesions (arrowhead).

copper excretion after admission. The findings of a physical examination as well as abdominal and neurological findings were normal. The liver and spleen were not palpable. The laboratory data on admission showed slightly elevated liver enzymes [aspartate aminotransferase (AST) 45 U/L, alanine aminotransferase (ALT) 50 U/L, γ-glutamyl transpeptidase (GTP) 58 U/L) and low serum copper and ceruloplasmin levels, and the 24-hour urinary copper level was 49.2 μg (Table 2). Brain magnetic resonance imaging (MRI) showed normal findings, including for the basal ganglia. An abdominal ultrasound examination revealed mild fatty liver with multiple round hypoechoic lesions (Fig. 1). Although the findings were suggestive of mottled fatty liver, malignancies, such as hepatocellular carcinoma, could not be denied.

We performed ultrasound guided percutaneous liver biopsies with a 17-gauge needle for a hypoechoic lesion as well as a normal area in the liver. Sections showed slight chronic inflammatory infiltration in the portal area, mild bridging fibrosis and mild fatty changes in the normal area. Fatty change was detected in a few hepatocytes in the hypoechoic lesion and in 15% of hepatocytes in the normal area. No copper-binding protein or copper deposition was identified with orcein or rhodanine staining in either specimen. There was no evidence of cirrhosis or malignancy (Fig. 2). Iron-

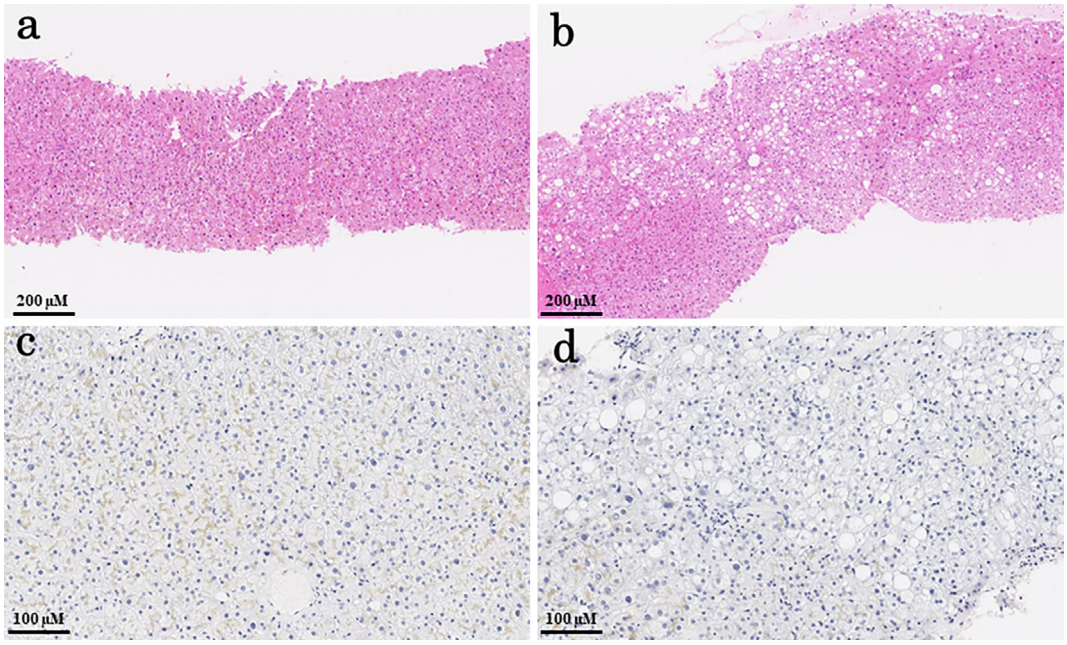

Figure 2. **The liver specimens were obtained from a hypoechoic lesion (a, c) and from a normal area (b, d). a: Hematoxylin and Eosin (H&E) staining showed almost normal findings. The specimen had few fatty changes in the hypoechoic lesion. b: H&E staining showed slight chronic inflammatory infiltration in the portal area, mild bridging fibrosis and mild fatty changes. The specimen had mild fatty changes in approximately 15% of hepatocytes in the normal area. c, d: Rhodanine staining showed no copper-binding protein or copper deposition. There was no evidence of cirrhosis or malignancy in either the hypoechoic lesion or the normal area.**

positive granules were observed in the hepatocytes on Prussian blue staining (data not shown). The hepatic copper contents were 281 μg/g dry weight (normal range: <40 μg/g dry weight) in the hypoechoic lesion and 306 μg/g dry weight in the normal area. Both copper contents were over 250 μg/g dry weight. There was no marked difference in the copper contents between the two lesions.

We changed the treatment from zinc acetate to D-penicillamine because of the high copper content in the liver. D-penicillamine was started at 400 mg/day. However, the serum γ-GTP level increased after the dose of D-penicillamine was increased to 600 mg/day. Therefore, we changed the chelator to trientine. The liver function improved after initiating trientine treatment. We gradually increased the dose of trientine, monitoring carefully to ensure that his liver function remained within the normal range (Fig. 3).

Discussion

Wilson disease is an autosomal recessive genetic disorder characterized by the accumulation of copper in various organs, because of ATP7B dysfunction caused by *ATP7B* mutations (1). Once the liver is saturated with copper, copper will overflow to other organs, such as the brain, eyes and various organs. Spillage copper, known as serum free copper, binds to albumin and various molecules. This free copper causes damage to a variety of organs, as free copper generates reactive oxygen species by Fenton reaction (3, 5). We revealed that copper-induced oxidative stress and endoplasmic reticulum (ER) stress were involved in the copper-induced cytotoxicity. Excess copper induced oxidative stress and ER stress even in a variety of cells expressing ATP7A (6).

Wilson disease is diagnosed based on serum ceruloplasmin levels, the measurement of urinary copper excretion, detection of Kayser-Fleischer rings, brain MRI findings, the measurement of intrahepatic copper content and the results of a genetic analysis of *ATP7B*. Nevertheless, we often have difficulty managing patients with this disease. The present patient was diagnosed with Wilson disease based on his intrahepatic copper content, genetic analysis results and detection of Kayser-Fleischer rings, and he was treated with D-penicillamine. He visited our hospital to have his liver function evaluated. The laboratory data showed low serum copper and ceruloplasmin levels and high serum ferritin levels. It is important to carefully monitor the status of copper and iron, as excessive copper chelating therapy induces anemia and iron deposition (7). In the present case, serum ferritin was high, so we changed the chelator to zinc acetate, thinking that the copper chelation was excessive. Furthermore, it is reported that patients with Kayser-Fleischer rings have a higher intrahepatic copper content than those without Kayser-Fleischer rings (8-10). In the present case, the disappearance of Kayser-Fleischer rings on admission suggested adequate copper chelation. However, his liver function test

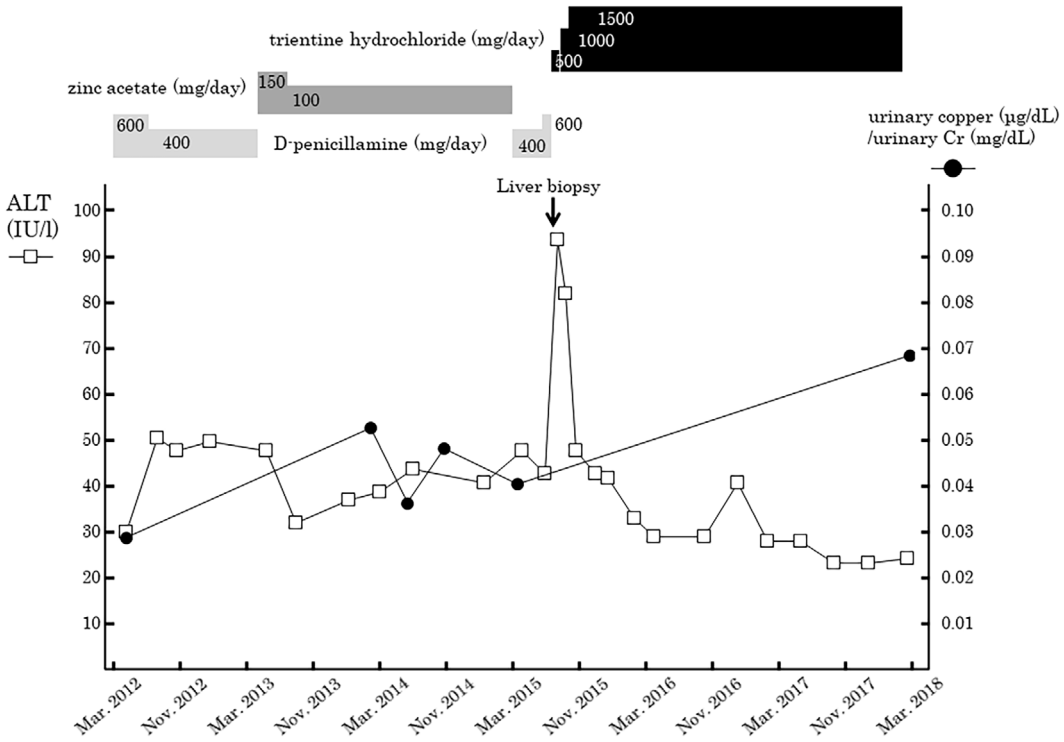

Figure 3. **Clinical course of the patient.**

did not normalize with zinc acetate. Therefore, we performed a liver biopsy, which revealed a high copper content, so we restarted copper chelating therapy. His liver function improved after resuming copper chelating therapy.

The therapeutic effects of Wilson disease treatment are generally evaluated by laboratory data, such as spot or 24-hour urinary copper, serum copper and ceruloplasmin levels. However, these parameters do not always accurately reflect the intrahepatic copper content in some patients. Thus, it is important to measure intrahepatic copper contents by liver biopsies in order to accurately evaluate the effectiveness of treatments. The reason why the urinary copper level was low and the Kayser-Fleischer rings had disappeared in the present case is unclear, but the patient's liver function improved after restarting adequate copper chelating therapy.

An ultrasound examination revealed multiple hypoechoic lesions. Liver biopsies revealed a mottled fatty liver. Pathological findings can show various types of features, including fatty change, acute hepatitis, chronic active hepatitis and cirrhosis in Wilson disease (2). Ultrasound, computed tomography and MRI findings of the liver reflect this wide range of pathological findings (11). However, it has been reported that one of the specific features in Wilson disease is multiple nodular lesions in the liver (11). The degree of fatty infiltration differed between the hypoechoic lesion and the normal area in the present case. It was reported that the intrahepatic copper content distribution is heterogenous in late-stage Wilson disease. Therefore, sampling errors when measuring the intrahepatic copper content can occur in cases of late-stage Wilson disease (1). In addition, it is reportedly difficult to detect copper in hepatocytes by copper staining in early-stage Wilson disease, as copper diffusely accumulates in the cytoplasm (12). Therefore, the measurement of the intrahepatic copper content by liver biopsies is important for the diagnosis of Wilson disease. We performed liver biopsies from a hypoechoic lesion and a normal area in the present case, and the intrahepatic copper contents were 281 and 306 μg/g dry weight, respectively. These findings indicated that a high copper content is present in this case of chronic Wilson disease, regardless of the degree of fatty change.

Conclusion

Wilson disease is one of the few treatable genetic disorders. When patients are adequately diagnosed and treated, the prognosis is good. However, laboratory data do not always accurately reflect the copper status in the body. It is important to measure the hepatic copper contents by a liver biopsy when the clinical course of the patient is unusual.

The authors state that they have no Conflict of Interest (COI).

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