Wilson disease is an autosomal recessive disorder of copper metabolism characterized by hepatic and/or neurological manifestations. This biochemical features of this disease are low serum ceruloplamine levels and high urinary copper excretion. Early diagnosis is very important to improve the prognosis of this disease. However, some patients revealed atypical biochemical findings. This study presents the efficacy of D-penicillamine challenge test for diagnosis of Wilson disease. Five patients and five normal controls were loaded 20mg/kg of D-penicillamine. Urinary copper / body weight (kg) ratio and/or urinary copper / creatinine ratio showed significant difference between Wilson disease patients and controls. The D-penicillamine challenge test will be useful for diagnosis of Wilson disease.

Keywords: Wilson disease, inborn error of copper metabolism, D-penicillamine, D-penicillamine challenge test, urinary copper excretion

Introduction

Wilson disease is an autosomal recessive disorder based on inborn error of copper metabolism. Copper is accumulated primarily in the liver, brain, cornea, kidney, and other organs. The copper accumulation is believed to result from the loss of ability to excrete copper via the bile due to a dysfunction of intracellular copper transport in the liver. The Wilson disease gene (ATP7B) encodes a putative copper-transporting P-type ATPase. Clinical features of this disease are liver cirrhosis, extra pyramidal signs and Kayser-Fleischer ring. The incidence is one in 35,000 to 45,000 in Japan\(^1\). Low serum ceruloplas-

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3) D-penicillamine challenge  
a) Wilson disease patients  
D-penicillamine was started 5-10 mg/kg/day and was increased to maintenance dose, step by step. And when they took 20 mg/kg/day of D-penicillamine, urinary copper levels were measured.

b) Normal controls  
Twenty milligram per kilogram of D-penicillamine was taken for one day.

c) D-penicillamine was given 2 or 3 times per day at least 1 hour before or 2 hours after meals.

4) Measurement of urinary copper excretion  
Daily urinary copper levels and urinary creatinine levels were measured. Then urinary copper / body weight (kg) ratio and urinary copper / creatinine ratio were calculated.

Results  
1) Daily urinary copper excretion during D-

![Graph](image1.png)  
*Fig. 1* Daily urinary copper excretion during D-penicillamine challenge test  
Lane 1 to 5 are Wilson disease patients, lane 1 is 6-year-old, 2 is 7-year-old, 3 is 8-year-old, 4 is 12-year-old and 6 is 18-year old. Lane 6 to 10 are normal controls, lane 6 is 12-year-old, 7 is 15-year-old and 8 to 10 are adult.

![Graph](image2.png)  
*Fig. 2* Urinary copper / body weight ratio during D-penicillamine challenge test  
Distributions of each lane are same as figure 1.
penicillamine challenge test
The daily urinary copper excretion of Wilson disease patients was 852.0 to 6291.2 µg/day and one of normal controls was 709.0 to 2080.0 µg/day (Fig. 1). There is no significant difference between patients and controls.

2) Urinary copper / body weight ratio
Urinary copper / body weight (kg) ratio of Wilson disease patients was 48.69 to 96.86 µg/kg/day (72.52 ± 19.20 µg/kg/day) and one of normal controls was 13.38 to 32.62 µg/kg/day (23.60 ± 6.70 µg/kg/day) (Fig.2). It was significant greater in patients with Wilson disease compared to controls.

3) Urinary copper / creatinine ratio
Urinary copper / creatinine ratio of Wilson disease patients was 2.27 to 4.86 µg/mg creatinine (3.55± 0.78 µg/mg creatinine) and one of normal controls was 0.45 to 1.32 µg/mg creatinine (0.86±0.25 µg/mg creatinine) (Fig.3). It was significantly higher in Wilson disease patients than in controls.

Discussion
The diagnostic criteria of Wilson disease are low serum ceruloplasmine levels (20mg/dl>) and high urinary copper excretion (100 µg/day<, 1.5 µg/kg< or 0.2 µg/mg creatinine<). However, some patients show atypical biochemical findings. The limitation of biochemical tests have been reported in several studies. Although measurement of hepatic copper content is still considered the golden standard, liver biopsy is invasive inspection. The diagnostic value of D-penicillamine challenge test was discounted by several authorities and different cut-off levels have been proposed. In previous studies, 1000 mg of D-penicillamine was ingested and cut-off level was established as 1600 µg/day. Although this protocol is a valuable diagnostic test for the symptomatic Wilson disease child, it is not effective for presymptomatic patients and adult patients.

In this study, 20 mg/kg/day of D-penicillamine was ingested for various ages of patients with Wilson disease and controls. One Wilson disease patient did not reach previously established cut-off value of 1600 µg/day and two normal controls reached it (Fig. 1). Daily urinary copper excretions followed by D-penicillamine challenge test were not significantly different between Wilson disease patients and normal controls (Fig. 1). Thus, this cut-off value may not help for diagnose of Wilson disease. However, urinary copper / body weight (kg) ratio and urinary copper / creatinine ratio of Wilson disease patients were significantly higher than normal controls (Fig. 2 & 3). D-penicillamine challenge test using these ratios will be useful as non-invasive testing for Wilson disease. Urinary copper / creatinine ratio must be measured for spot urine samples. If yes, this test can be performed for out-patients. It may be convenient for patients. The authors would like to propose temporary cut-off value of 35 µg/kg/day and/or 2.0 µg/mg creatinine for D-penicillamine 20 mg/kg/day challenge test. In the future, many and variable kinds of Wilson disease patients (especially for asymptomatic type patients) and control subjects (for example another live disease) should be investigated. Then, the efficacy of our protocol must be con-
firmed and final cut-off value should be established.

References


