Is it necessary to re-evaluate diagnostic criteria for Wilson disease in children?

**ABSTRACT**

**Background/Aims:** The differential diagnosis of Wilson Disease (WD) is challenging, especially in children, because liver copper levels may also increase in other chronic liver diseases with bile stasis. The aim of this study is to determine urine and liver copper cut-off values to differentiate WD from other chronic liver diseases (non-WD, NWD) in children.

**Materials and Methods:** Seventy-six patients participated in the study, 35 with WD and 41 with NWD. The two groups were divided into two subgroups according to the presence of cholestasis. At the time of diagnosis, age, sex, biochemical test results, serum ceruloplasmin, baseline 24-h urinary copper levels, liver biopsy histological findings, liver copper levels, and Child-Pugh scores were obtained from medical records. Copper content in liver tissue and copper levels in urine were measured by atomic absorption spectrometry. Cut-off values for differentiation of WD from NWD were determined by receiver operating characteristic (ROC) analysis.

**Results:** A liver copper cut-off value of 98 µg/g indicated WD with 91% sensitivity and 65.4% specificity (area under the curve =0.838, 95% CI: 0.749-0.927). A 24-h urinary copper cut-off value of 67.5 µg/24h indicated WD with 85% sensitivity and 71% specificity (area under the curve =0.843, 95% CI: 0.752-0.934).

**Conclusion:** In this study of pediatric chronic liver disease patients, copper cut-off values for distinguishing WD differed substantially from those used for diagnosis. A larger scale study is warranted to re-evaluate liver copper and 24-h urinary copper cut-offs for children with suspected WD.

**Keywords:** Liver copper, liver disease, children

**INTRODUCTION**

The homeostatic regulation of copper depends on a balance between liver storage, biliary excretion, and incorporation into the transport protein ceruloplasmin (1-4).

Wilson disease (WD) is an autosomal recessive hereditary disorder caused by mutation of the *ATP7B* gene, which encodes a P-type copper transporting ATPase (5-7). This mutation alters the hepatic excretion of copper, with consequent accumulation, particularly in the liver, central nervous system, cornea, and kidney (8). A low ceruloplasmin level (<20mg/dL), baseline hypercupriuria (>100 µg/24h), elevated urinary copper excretion following oral administration of D-penicillamine, elevated liver copper content (>250 µg/g dry weight), and mutation analysis of the *ATP7B* gene are helpful for diagnosis of WD (9-11). However, hepatic copper content >250 µg/g in the absence of cholestasis is considered the best available diagnostic index (12,13).

Liver and urinary copper levels may also increase in other chronic liver diseases with bile stasis, obfuscating the differential diagnosis of WD. Unfortunately, there are insufficient data on hepatic copper levels in children with...
Nutrition Department to investigate the etiology of chronic liver diseases other than WD. The aim of this study is to determine urine and liver copper cut-off values to differentiate WD from other chronic liver diseases in children.

MATERIALS AND METHODS

This retrospective study was conducted in children referred to Baskent University Pediatric Gastroenterology Hepatology and Nutrition Department to investigate the etiology of chronic hepatic disease. The patients underwent percutaneous liver biopsy for histological diagnosis and determination of liver copper were included in this study. At the time of diagnosis, age, sex, biochemical test results, serum ceruloplasmin, baseline 24-h urinary copper concentration, liver biopsy histological findings, liver copper levels, and Child-Pugh scores were obtained from medical records.

Copper content in dried liver tissue was measured by atomic absorption spectrometry (Atomic Absorption Spectrophotometer AA-6701F, Shimadzu, Japan). The presence of cholestasis was based on histological findings in liver biopsy samples and biochemical findings such as high serum direct bilirubin levels. Urinary samples were collected in an acid-washed plastic metal-free container and copper concentration measured by atomic absorption spectrometry (Atomic Absorption Spectrophotometer AA-6701F, Shimadzu, Japan). Serum ceruloplasmin concentration (normal range, 20-60 mg/dL) was measured by the immunoturbidimetric method (Roche/Hitachi Modular Analytics P, Tokyo, Japan). Diagnosis of WD (n=35) was supported by the identification of two disease-causing mutations or homozygosity for a single disease-causing mutation. In addition, WD diagnostic scores were calculated according to the classification of Ferenci et al. (14).

Patients with cryptogenic cirrhosis and chronic hepatitis were included in the NWD group if their WD diagnosis scores were 0 or 1 (i.e., WD unlikely). The underlying diseases in the NWD group (n=41) were as follows: cryptogenic cirrhosis (n=18), chronic hepatitis (n=4), non-alcoholic steatohepatitis (n=4) congenital hepatic fibrosis (n=2), progressive familial intrahepatic cholestasis (PFIC) (n=3), portal vein thrombosis (n=1), tyrosinemia (n=4), autoimmune hepatitis (n=4), and glycogen storage disease (n=1).

Patients with signs of systemic diseases or infections at the time of diagnosis and those who had undergone prior therapy with chelating agents or zinc were excluded from the study. The study was approved by the local ethics committee (Study no: KA0976). Written informed consent was obtained from patient's guardians.

We used SPSS version 16.0 (SPSS Inc, Chicago, IL) software for statistical analyses. Pair-wise differences in continuous variables were assessed by the Mann-Whitney U-test. Chi-square and Fisher exact tests were used to compare categorical data. A p<0.05 was considered significant. Correlations among the data were evaluated using Spearman's correlation. We used Receiver Operating Characteristic (ROC) analysis to determine optimal cut-off values for liver and urinary copper.

RESULTS

Seventy-six patients, thirty-five with WD as indicated by ATP7B mutation analysis and and forty-one with NWD participated in the study. The two groups were divided into two subgroups according to the presence of cholestasis. Mean age was 122.4±45.3 months (range: 8-16.5 years) in the WD group and 138±42.7 months (range: 7-17.6 years) in the NWD group. There was no statistical difference in mean age or gender ratio between WD and NWD groups. In addition, the proportion of patients with cholestasis, mean Child-Pugh scores, and mean direct bilirubin concentration did not differ between WD and NWD groups (Table 1).

Liver copper and cholestasis

Mean liver copper level was significantly higher in the WD group compared to the NWD group (p=0.001). Liver copper levels were not correlated with age at liver biopsy in either group. The mean serum direct bilirubin concentration in NWD patients with cholestasis was substantially higher than in NWD patients without cholestasis (13.4±8.7 mg/dL vs. 0.7±0.72 mg/dL). There was no significant difference in mean liver copper content between WD patients with cholestasis and those without cholestasis (Table 2). In contrast, the liver copper content was significantly higher in cholestatic NWD patients compared to non-cholestatic NWD patients (p=0.033). In NWD, a positive correlation was detected between direct bilirubin and both liver copper (r=0.13, p=0.017) and Child-Pugh score (p=0.21, r=0.093).

Table 1. Demographic characteristics and laboratory results from pediatric Wilson disease (WD) and non-Wilson disease (NWD)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NWD</th>
<th>WD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender Female</td>
<td>16 (39%)</td>
<td>15 (42.9%)</td>
<td>p=0.735</td>
</tr>
<tr>
<td>Male</td>
<td>25 (61%)</td>
<td>20 (57.1%)</td>
<td></td>
</tr>
<tr>
<td>Age (months)</td>
<td>138±42.7</td>
<td>122±45.3</td>
<td>p=0.129</td>
</tr>
<tr>
<td>Presence of cholestasis</td>
<td>20 (48.8%)</td>
<td>19 (54.3%)</td>
<td>p=0.632</td>
</tr>
<tr>
<td>Child-Pugh scores</td>
<td>7.2±1.9</td>
<td>7.6±2.2</td>
<td>p=0.226</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>214.7±302.6</td>
<td>205.9±191.3</td>
<td>p=0.463</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>201.5±341.1</td>
<td>144.6±103.9</td>
<td>p=0.613</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dL)</td>
<td>6.9±8.8</td>
<td>6.6±10.3</td>
<td>p=0.606</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>3.6±0.8</td>
<td>3.8±0.7</td>
<td>p=0.749</td>
</tr>
<tr>
<td>INR</td>
<td>1.6±0.6</td>
<td>1.9±1</td>
<td>p=0.026</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase; ALT: alanin-aminotransferase; INR: international normalized ratio
The proportion of patients with liver copper concentration >250 µg/g was significantly higher in the WD group than the NWD group (65.7% vs. 24.4%, p=0.001). Twelve out of 35 patients in the WD group (34.3%) had a liver copper level <250 µg/g. Liver copper level was >250 µg/g in 10 patients in NWD (24.4%) (Table 3). Six of the 10 NWD patients with cholestasis had cryptogenic cirrhosis, and their median liver copper level was 371.5 µg/g (300-1980 µg/g). The diagnoses of the other four NWD patients with cholestasis were congenital hepatic fibrosis (n=1) and cryptogenic cirrhosis (n=3), and their median liver copper level was 337 µg/g (257-440 µg/g).

Receiver operating characteristic analysis of liver copper levels using a cut-off value of 98 µg/g yielded 91% sensitivity and 65.4% specificity for distinguishing WD from NWD (area under the curve of 0.838, 95% CI: 0.749-0.927, Figure 1). Liver copper was <98 µg/g in only 3 of 35 WD patients (8.5%) and >98 µg/g in only 15 of 41 NWD patients (36.6%) (Table 3). Decreasing the liver copper content cut-off value from 250 µg/g to 98 µg/g increased the sensitivity from 65% to 91% and decreased the specificity from 77% to 65%.

**Urinary copper**

Mean basal 24-h urinary copper excretion was significantly higher in the WD group compared to the NWD group (p=0.0001). In the NWD group, mean 24-h urinary copper levels were significantly higher in the cholestatic subgroup (p=0.012) (Table 2). There was a positive correlation between direct bilirubin and 24-h urinary copper levels in both the NWD and WD group (NWD: p=0.002, r=0.477; WD: p=0.008, r=0.439).

A 24-h urinary copper cut-off value of 67.5 µg/24h distinguished WD from NWD with 85% sensitivity and 71% specifi-
There was a positive correlation between ceruloplasmin levels and bilirubin levels (r=0.383, p=0.02) in WD patients. In contrast, there was no difference in the relationship between cholestasis and urinary copper excretion in children with WD. However, only 65.7% of the patients with cholestasis had urinary copper levels >250 µg/g. Therefore, as anticipated, ceruloplasmin levels were significantly lower in patients with cholestasis. Further studies are required to examine the relationship between cholestasis and urinary copper levels in pediatric NWD patients.

As anticipated, ceruloplasmin levels were significantly lower in WD patients than in NWD patients. However, 29% of NWD patients had liver copper levels >250 µg/g compared to only 1.4% of children and adults with non-cholestatic liver disease (sensitivity: 83.3%; specificity: 98.6%). ROC analysis suggested <75 µg/g as the most useful liver copper cut-off value to exclude WD (sensitivity 96.5% and specificity 95.4%). Moreover, they found that liver copper levels were not correlated with age or with the severity of liver histological abnormalities in a total of 112 WD patients with or without ATP7B mutations (5). Nicastro et al. (6) found that only 2% of children with WD had liver copper levels <75 µg/g. Similarly, Rosentcrantz and Schilsky recommended 75 µg/g as the threshold hepatic copper concentration for the differential diagnosis of WD (24). Thus, a cut-off substantially lower than 250 µg/g may still effectively distinguish WD from NWD in children, particularly for patients without cholestasis. In our study, liver copper level was >250 µg/g in 10 NWD patients (24.5%), 6 out of the 10 patients with high liver copper levels had cholestasis.

DISCUSSION

The conventional criteria for distinguishing WD are based on adult values, making differential diagnosis of WD an especially challenging task in children. Liver copper content >250 µg/g is a useful parameter, but a lower value does not exclude WD. Moreover, liver copper may increase above 250 µg/g, a conventional cut-off value, in other chronic liver diseases with bile stasis. Göksu et al. (15) reported high copper concentrations in liver samples obtained from infants with biliary atresia at autopsy and Bayliss et al. (16) reported high copper levels in liver specimens obtained from biliary atresia patients at the time of radical surgery. Elevated hepatic copper levels are seen in cholestatic liver diseases such as primary biliary cirrhosis, extrahepatic biliary obstruction, cryptogenic cirrhosis, and hepatitis in studies on humans and animals (17-23). In our study, mean liver copper levels were higher in WD patients than in patients with non-WD liver diseases. However, only 65.7% of the patients with WD had liver copper levels >250 µg/g, while 91.5% had liver copper levels >98 µg/g compared to only 36.6% of NWD patients. In addition we found that, liver copper levels in both WD and NWD did not correlate directly with age at liver biopsy.

Ferenci et al. (18) found that 83.3% of WD patients had liver copper levels >250 µg/g, compared to only 1.4% of children and adults with non-cholestatic liver disease (sensitivity: 83.3%; specificity: 98.6%). ROC analysis suggested <75 µg/g as the most useful liver copper cut-off value to exclude WD (sensitivity 96.5% and specificity 95.4%). Moreover, they found that liver copper levels were not correlated with age or with the severity of liver histological abnormalities in a total of 112 WD patients with or without ATP7B mutations (5). Nicastro et al. (6) found that only 2% of children with WD had liver copper levels <75 µg/g. Similarly, Rosentcrantz and Schilsky recommended 75 µg/g as the threshold hepatic copper concentration for the differential diagnosis of WD (24). Thus, a cut-off substantially lower than 250 µg/g may still effectively distinguish WD from NWD in children, particularly for patients without cholestasis. In our study, liver copper level was >250 µg/g in 10 NWD patients (24.5%), 6 out of the 10 patients with high liver copper levels had cholestasis.

Basal urinary copper excretion in children with WD is lower than the widely accepted cut-off value of 100 µg/24h (25). In adult studies, the sensitivity of the 100 µg/24h cut-off has ranged from 59% to 88% (26-28). One report, however, reported a reasonable sensitivity of 68% at 40 µg/24h (27). In pediatric series, urinary copper levels exceeded 100 µg/24h in 81% to 94% of cases (29-31). A basal cupriuria cut-off value of 63.5 µg/24h yielded sensitivities of approximately 95% and 70% in symptomatic and asymptomatic children, respectively (30,32). We found 67.5 µg/24h to be the best cut-off value for children. Only 8.5% of WD patients had urinary copper levels lower than 67.5 µg/24h compared to 70.7% of NWD patients. Twenty-four-hour urinary copper levels were significantly higher in NWD patients with cholestasis. Further studies are required to examine the relationship between cholestasis and urinary copper levels in pediatric NWD patients.

As anticipated, ceruloplasmin levels were significantly lower in WD patients than in NWD patients. However, 29% of NWD patients had ceruloplasmin levels <20 mg/dL and eight patients with WD (22%) had ceruloplasmin levels >20 mg/dL. Therefore, we could not define a cut-off value for ceruloplasmin likely to help differentiate WD from other chronic liver diseases in children.

The main limitation of this study is that liver copper measures are based on a single biopsy sample. Thus, variation may reflect regional differences in hepatic copper distribution, especially in the cirrhotic liver. However, it is difficult to take multiple or larger biopsy samples from children.

Table 4. Distribution of patients by 24-h urinary copper levels

<table>
<thead>
<tr>
<th>24-h urinary copper levels (µg/24-h)</th>
<th>&lt;67.5</th>
<th>&gt;67.5</th>
<th>&lt;100</th>
<th>&gt;100</th>
</tr>
</thead>
<tbody>
<tr>
<td>WD</td>
<td>4 (11.4%)</td>
<td>31 (88.6%)</td>
<td>7 (20%)</td>
<td>28 (80%)</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>2 (10.5%)</td>
<td>17 (89.4%)</td>
<td>3 (15.8%)</td>
<td>16 (84.2%)</td>
</tr>
<tr>
<td>Non-cholestatic</td>
<td>2 (12.5%)</td>
<td>14 (87.5%)</td>
<td>4 (25%)</td>
<td>12 (75%)</td>
</tr>
<tr>
<td>NWD</td>
<td>29 (70.7%)</td>
<td>13 (29.3%)</td>
<td>31 (75.6%)</td>
<td>10 (24.4%)</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>11 (55.0%)</td>
<td>10 (45.0%)</td>
<td>12 (60%)</td>
<td>8 (40%)</td>
</tr>
<tr>
<td>Non-cholestatic</td>
<td>18 (85.7%)</td>
<td>3 (14.3%)</td>
<td>19 (90.5%)</td>
<td>2 (9.5%)</td>
</tr>
</tbody>
</table>

WD: Wilson disease

Ceruloplasmin

Mean ceruloplasmin concentration was significantly lower in the WD group compared to the NWD group (16.2±10.9 mg/dL vs. 28.1±12.2 mg/dL, p=0.001) and lower in the WD cholestatic subgroup compared to the non-cholestatic WD subgroup (11.5±6.9 vs. 19.6±12.2, p=0.002). There was a positive correlation between ceruloplasmin and bilirubin levels (r=0.383, p=0.02) in WD patients. In contrast, there was no difference in ceruloplasmin concentration between cholestatic and non-cholestatic NWD subgroups (28.2±96.4 mg/dL vs. 28.1±14.5 mg/dL, p>0.05). Unexpectedly, 14 patients in the NWD group (34.1%) had ceruloplasmin levels <20 mg/dL (mean of 15.9±3.7 mg/dL). Eight patients in WD group (22%) had mean ceruloplasmin levels >20 mg/dL (mean of 32.5±10.6 mg/dL).
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We conclude that the hepatic and urinary copper cut-off values conventionally used for WD diagnosis are not effective for the differential diagnosis of WD in children. Rather, lower liver and urine copper levels had greater sensitivity for differential diagnosis. To accurately distinguish WD from other chronic liver diseases with or without cholestasis in children, new threshold values must be defined and confirmed.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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