Urinary 24-hour copper excretion at the time of diagnosis in children with Wilson's disease

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Abstract

The optimal cut-off value of 24-hour (h) urinary copper (Cu) levels to identify Wilson's disease (WD) has not been widely studied in children. In sixty-six children with confirmed WD and 88 children without WD, 24-h urinary excretion of Cu at the time of diagnosis was studied. The receiver operating characteristic (ROC) curves revealed that the optimal cut-off value of urinary Cu to identify WD was 70 mcg [area under the curve (AUC) = 0.894] with a sensitivity and specificity of 81.8% and 89.8%, respectively. When the serum ceruloplasmin level was <20 mg/dl and the 24-h urinary excretion of Cu was >70 mcg, the sensitivity was 75.8%, and the specificity was 97.7%. After the exclusion of cholestatic patients, the ROC curves revealed that the optimal cut-off value for 24-h urinary Cu excretion was 55 mcg (AUC = 0.910) with a sensitivity and specificity of 83.3% and 90.3%, respectively. When the ceruloplasmin level was <20 mg/dl and the 24-h urinary Cu excretion was >55 mcg, the sensitivity and specificity were 77.3% and 98.4%, respectively.

A 24-h urinary Cu level of >70 mcg plus a ceruloplasmin level of <20 mg/dl in the patients, and a 24-h urinary Cu level of >55 mcg plus a ceruloplasmin level of <20 mg/dl in non-cholestatic patients exhibited the highest specificity and the highest positive and negative predictive values to identify WD in children. (Acta gastroenterol. belg., **2018**, 81, **410-414**).

Keywords : 24-hour urinary copper, ceruloplasmin, screening practices, receiver operating characteristic curves.

Introduction

Wilson's disease (WD) is an autosomal recessive disease characterized by reduced biliary copper excretion and deposition of copper (Cu) in the tissues, particularly in the liver and neurological system. Its prevalence is 1 in 30,000 in the general population (1). Diagnosis and treatment of WD at an early stage can avoid severe hepatic and neurological complications. The symptoms and clinical findings caused by Cu deposition develop gradually; therefore, WD is difficult to diagnose at an early stage, particularly in pediatric patients. Hepatic and/or neurological signs and symptoms, Kayser-Fleischer (KF) rings, low serum ceruloplasmin concentrations, and increased urinary Cu excretion are the major diagnostic features of WD (2).

There is not a single diagnostic test apart from genetic testing that can exclude or confirm WD. The diagnosis by genetic testing is, however, expensive and not universally available. Because various mutations and polymorphisms of ATP7B have been associated with WD, it is difficult to assess all mutations. A combination of clinical features and laboratory parameters is used to establish the diagnosis; however, none of them is pathognomonic of WD. KF rings are often absent in WD and can be seen in other chronic liver diseases (2,3). Serum ceruloplasmin concentrations may be normal in WD, and low concentrations can also be seen in protein malnutrition, protein-losing disorders, or severely impaired hepatic function (2,3). Among all diagnostic tests, the most simple and sensitive test is the 24-hour (h) urinary Cu level, which reflects the amount of nonceruloplasmin-bound Cu in the circulation (4,5).

Although 24-h urinary Cu excretion <40 mcg is considered normal, and excretion of >100 mcg is considered a diagnostic criterion for WD in adults (6), the diagnostic levels of urinary Cu for WD have not been widely studied in children (7,8). Here, we studied 24-h urinary Cu excretion in combination with serum ceruloplasmin in children with WD at the time of diagnosis.

Methods

The study was conducted at the Pediatric Gastroenterology and Hepatology division of Gazi University Medical Faculty Hospital, a tertiary-care hospital, between January 2004 and December 2013. One hundred and fifty-four children (2-18 years) were enrolled in the study. We retrospectively reviewed the 24-h urinary Cu excretion of children at the time of diagnosis with WD, children with other chronic liver disease, and healthy children who were screened for WD due to an affected sibling or family member or a preliminary diagnosis.

The diagnosis of WD in the patients was based on the presence of liver disease and at least two of the following criteria according to the scoring system developed at the 8th International Meeting on WD, Leipzig 2001 (9).

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1. positive family history,
2. low serum ceruloplasmin (<20 mg/dL),
3. elevated liver Cu (>250 mcg/g dry weight),
4. elevated baseline 24-h urinary Cu excretion (>100 mcg/24 h),
5. elevated 24-h urinary Cu excretion following administration of two, 500-mg doses of penicillamine (>1575 mcg/24 h),
6. presence of KF rings and
7. Coombs' negative hemolytic anemia.

Additionally, brain MRI was performed in suspected neurological WD patients.

We studied serum ceruloplasmin, and 24-h urinary Cu levels, and an eye examination was performed to identify a KF ring. If the patient had liver disease accompanied by a low ceruloplasmin level and/or a high urinary copper level and/or a KF ring in addition to a family member diagnosed with WD, the diagnosis of WD was made. In these patients, no other diagnostic tests such as a penicillamine challenge test or a liver biopsy were performed. If there was no WD patient in the family, a penicillamine challenge test and liver biopsy with measurement of the Cu content in the specimen was performed. We diagnosed WD according to the scoring system. We were not able to perform mutation analysis in our patients because ATP7B mutation is not analyzed in our hospital laboratories (10).

Urine samples were collected in a plastic container, and the patients were instructed not to rinse the container with tap water, which might have high Cu content. All patients' serum creatinine levels and creatinine clearance by Schwartz estimate were normal, at the time of the urine sample collection. Urinary Cu levels were determined by atomic absorption spectrophotometry, and serum ceruloplasmin was measured by immunoturbidimetry.

Statistical analysis

Descriptive analysis was performed for demographic and clinical characteristics of the patients. Mann-Whitney U test was used for comparison of continuous variables between two groups, chi-square test was used for comparison of ratios between the groups, and Spearman test was used for correlation analysis. The diagnostic accuracy of the 24-h urinary Cu level was assessed by receiver operating characteristic (ROC) curves. Sensitivity, specificity, positive and negative likelihood ratio, and positive and negative predictive value were calculated for all cut-off levels with 95% confidence intervals (CI). All the cut-off values were designated as the probability of a true positive (sensitivity) and a true negative (specificity). Statistical analysis was performed with SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). All the statistical tests used were two-tailed, and statistical significance was set at a *p*-value <0.05.
The study was approved by the local research ethics committee (protocol no: 2015-11/187).

Results

A total of 154 children, including 66 children, aged 2-18 years (30 male) with confirmed WD and 88 children, aged 3-18 years (47 male) without WD, were evaluated retrospectively. The non-WD group included healthy children, children screened for WD due to an affected sibling or family member (n = 19) and children having chronic liver diseases [autoimmune hepatitis (n = 22) ; elevated liver enzymes and/or hepatomegaly with unknown etiology, cryptogenic cirrhosis (n = 17) ; familial intrahepatic cholestasis (n = 7) ; other chronic liver diseases, e.g., metabolic, immunologic, anatomic, etc. (n = 23)]. Demographic features, laboratory findings, serum ceruloplasmin levels, and 24-h urinary Cu levels are shown in Table 1. We found no significant differences between the WD patients and patients without WD in terms of age, sex, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), total and direct bilirubin, albumin levels, and prothrombin time/international normalized ratio (PT/INR). The 24-h urinary Cu levels were significantly higher and ceruloplasmin levels were significantly lower in the WD group compared with the non-WD liver disease and healthy control groups (Table 2). Urinary copper levels were significantly correlated with GGT and total bilirubin levels ($r = 0.26$, $p = 0.001$; $r = 0.21$, $p = 0.013$, respectively) but not with age, gender, and other liver function tests ($p > 0.05$).

The ROC curves revealed that the optimal cut-off value of 24-h urinary Cu level to diagnose WD was 70 mcg [area under the curve (AUC) = 0.894, ($p < 0.0001$) (95% CI: 0.841–0.947)] with a sensitivity of 81.8%, specificity of 89.8%, positive predictive value of 85.7, and negative predictive value of 86.8 (Fig. 1). When the diagnostic value was >100 mcg, the sensitivity and specificity were 69.7% and 92.1%, respectively, and positive and negative predictive values were 86.8 and

Table 1. — **WD and non-WD group's demographic features and the serum ceruloplasmin levels and 24-h urine Cu levels**

	WD Patients (n=66)	Non-WD Patients (n=88)	P-value
Age, years	10 (7-13)	9.5 (7-13)	0.891
Sex (male) n (%)	30 (45.5)	47 (53.4)	0.329
AST, U/L	99 (48-199)	72 (42-257)	0.667
ALT, U/L	110 (37-247)	93 (29-214)	0.765
GGT, U/L	60 (28-129)	40 (18-116)	0.070
Total bilirubin, mg/dl	0.9 (0.5-2)	0.6 (0.4-3.1)	0.425
Direct bilirubin, mg/dl	0.3 (0.1-1)	0.3 (0.1-2.1)	0.980
Albumin, g/dl	4.3 (3.5-4.6)	4.3 (3.8-4.5)	0.780
PT, sec.	14 (12-19)	14 (12-16)	0.602
INR	1.1 (1-1.6)	1.2 (1-1.4)	0.645
Ceruloplasmin, mg/dl	8 (7-10)	27 (24-35)	**<0.0001**
24-h urine Cu level, mcg	174 (90-482)	17 (9-39)	**<0.0001**

Continuous variables are presented as median (25th-75th percentile).
Wilson's disease, WD ; aspartate aminotransferase, AST ; alanine amino-transferase, ALT ; gamma-glutamyl transferase, GGT ; prothrombin time, PT ; international normalization ratio, INR.

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Table 2. — **WD, healthy control and non-WD liver disease group's the serum ceruloplasmin levels and 24-h urine Cu levels**

		WD (n=66)	Healthy control (n=17)	Non-WD liver disease (n=71)
Ceruloplasmin, mg/dl	Median (IQR)	8 (7-10)*#	26 (24-29)*	28 (24-36)#
	Min-Max	2-28	19-46	3-70
24-h urine Cu level, mcg	Median (IQR)	174 (90-482)*#	21 (9-36)*	16 (8-41)#
	Min-Max	8-1924	2-135	1-602

Continues variables are presented as median (25th-75th percentile). *p value is <0.0001 between WD and healthy control group. #p value is <0.0001 between WD and non-WD liver disease.

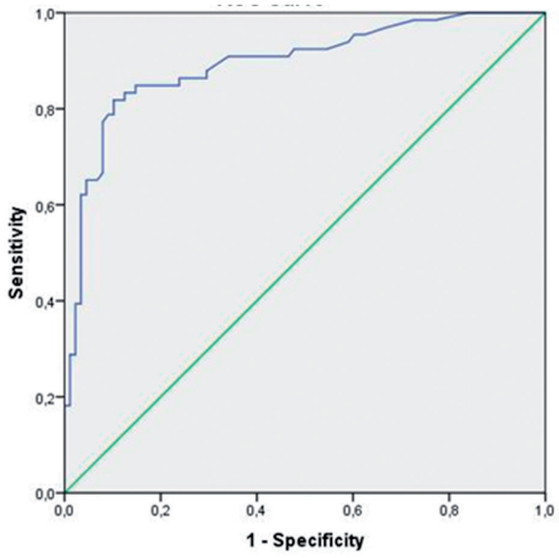

Fig. 1. — ROC curve for 24-h urine Cu level, area under the curve=0.894 (p <0.0001) (95% CI: 0.841-0.947). Receiver operating characteristic, ROC.

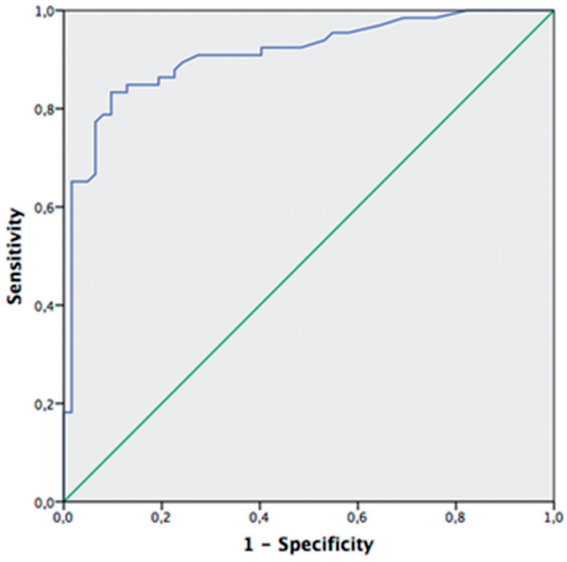

Fig. 2. — ROC curve for 24-h urine Cu level, area under the curve=0.910 (p <0.0001) (95% CI: 0.858-0.962) after exclusion of cholestatic patients. Receiver operating characteristic, ROC

80.2, respectively. For serum ceruloplasmin levels <20 mg/dl and 24-h urinary Cu levels >70 mcg, the sensitivity and specificity were 75.8% and 97.7%, respectively, and positive and negative predictive values were 96.2 and 84.3, respectively. Finally, for serum ceruloplasmin levels <20 mg/dl and 24-h urinary Cu levels >100 mcg, the sensitivity and specificity were 65.2% and 97.7%, respectively, and positive and negative predictive values were 95.6 and 78.9, respectively, for WD (Table 3).

After exclusion of the cholestatic patients, the ROC curves revealed that the optimal cut-off value of 24-h urinary Cu level to diagnose WD was 55 mcg [AUC = 0.910, (p < 0.0001) (95% CI: 0.858-0.962)] with a sensitivity and specificity of 83.3% and 90.3%, respectively, and positive and negative predictive values of 90.2 and 83.6, respectively (Fig. 2). With a 24-h urinary Cu cut-off level of >100 mcg, the sensitivity and specificity were 69.7% and 92.1%, respectively, and positive and negative predictive values were 92.0 and 74.4, respectively. The combination of a ceruloplasmin level of <20 mg/dl and 24-h urinary Cu level of >55 mcg was associated with a sensitivity and specificity of 77.3% and 98.4%, respectively, and positive and negative predictive values of 98.1 and 80.3, respectively. The combination of a ceruloplasmin level of <20 mg/dl and a 24-h urine Cu level of >100 mcg was associated with a sensitivity and specificity of 65.2%and 98.4%, respectively, and the positive and negative predictive values were 97.7 and 72.6, respectively (Table 4).

Discussion

Our aim was to identify WD patients from a group of children who were screened for WD based on 24-h urinary Cu excretion. We evaluated all children with either acute or chronic symptomatic liver disease or liver failure with WD. In our study, the most distinctive cut-off value of 24-h urinary Cu for the diagnosis of WD in children was 70 mcg, and the diagnostic accuracy of the 24-h urinary Cu level increased when combined with an assessment of the serum ceruloplasmin level. After exclusion of the cholestatic patients, the most distinctive cut-off value of 24-h urinary Cu for the diagnosis of WD in children was 55 mcg. In addition, we demonstrated that the urinary Cu levels were significantly correlated with GGT and total bilirubin levels. A similar positive correlation was shown in a previous publication (11).

The 24-h urinary Cu excretion has been demonstrated to be one of the most sensitive tests for the diagnosis of WD (4,5). A 24-h urinary Cu level of >100 mcg is acceptable as a diagnostic criterion for WD in adults. However, in 16%–23% of WD patients, 24-h urinary Cu

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Table 3. — **Sensitivity, specificity, positive and negative likelihood ratio, and positive and negative predictive values for the 24-h urine Cu with/without serum ceruloplasmin levels**

	24-h urine Cu level			**Ceruloplasmin <20mg/dl + 24-h urine Cu level**		
	>40mcg	>70mcg	>100mcg	>40mcg	>70mcg	>100mcg
Sensitivity, % (95% CI)	86.4 (75.7- 93.6)	81.8 (70.4-90.2)	69.7 (57.2-80.4)	80.3 (68.7-89.1)	75.8 (63.6-85.5)	65.2 (52.4-76.5)
Specificity, % (95% CI)	76.1 (65.9-84.6)	89.8 (81.5-95.2)	92.1 (84.3-96.7)	94.3 (87.2-98.1)	97.7 (92.0-99.7)	97.7 (92.0-99.7)
+ LR, (95% CI)	3.6 (2.5-5.3)	8 (4-15)	8.8 (4.2-18.2)	14.1 (6.0-33.4)	33.3 (8.4-132.1)	28.7 (7.2-114.1)
- LR, (95% CI)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.3 (0.2-0.5)	0.2 (0.1-0.3)	0.3 (0.2-0.4)	0.4 (0.3-0.5)
PPV, % (95% CI)	73.1 (61.8-82.5)	85.7 (74.6-93.3)	86.8 (74.7-94.5)	91.4 (81.0-97.1)	96.2 (86.8-99.5)	95.6 (84.9-99.5)
NPV, % (95% CI)	88.2 (78.7-94.4)	86.8 (78.1-93.0)	80.2 (71.1-87.5)	86.5 (78.0-92.6)	84.3 (75.8-90.8)	78.9 (70.0-86.1)

Positive likelihood ratio, + LR ; negative likelihood ratio, - LR ; positive predictive value, PPV ; negative predictive value, NPV.

Table 4. — **Sensitivity, specificity, positive and negative likelihood ratio, positive and negative predictive values for the 24-h urine Cu with /without serum ceruloplasmin levels after exclusion of cholestatic patients**

	24-h urine Cu level			**Ceruloplasmin <20mg/dl + 24-h urine Cu level**		
	>40mcg	>55mcg	>100mcg	>40mcg	>55mcg	>100mcg
Sensitivity, % (95% CI)	86.4 (75.7- 93.6)	83.3 (72.1-91.4)	69.7 (57.2-80.4)	80.3 (68.7-89.1)	77.3 (65.3-86.7)	65,2 (52.4-76.5)
Specificity, % (95% CI)	80.7 (68.6-89.6)	90.3 (80.1-96.4)	92.1 (84.3-96.7)	95.2 (86.5-99)	98.4 (91.3-100)	98.4 (91.3-100)
+ LR, (95% CI)	4.5 (2.7-7.5)	8.6 (4-18.6)	10.8 (4.1-28.3)	16.6 (5.5-50.4)	47.9 (6.8-336.2)	40.4 (5.7-284.5)
- LR, (95% CI)	0.2 (0.1-0.3)	0.2 (0.1-0.3)	0.3 (0.2-0.5)	0.2 (0.1-0.3)	0.2 (0.2-0.4)	0,4 (0.3-0.5)
PPV, % (95% CI)	82.6 (71.6-90.7)	90.2 (79.8-96.3)	92 (80.8-97.8)	94.6 (85.1-98.9)	98.1 (89.7-100)	97.7 (88.0-99.9)
NPV, % (95% CI)	84.8 (73.0-92.8)	83.6 (72.5-91.5)	74.4 (63.2-83.6)	81.9 (71.1-90.0)	80.3 (69.5-88.5)	72.6 (61.8-81.8)

Positive likelihood ratio, + LR ; negative likelihood ratio, - LR ; positive predictive value, PPV ; negative predictive value, NPV.

levels were found to be less than 100 mcg at the time of WD diagnosis (12). Cu accumulation generally takes time to clinically present as WD in children, particularly in young children (13). A correlation between the type of mutation and the urinary Cu concentration has not been described (7). Although some previous studies have shown a correlation between age and basal urinary Cu concentration at the time of diagnosis of WD (7,13,14), we did not find such correlation in this study. Nicastro et al. (13) demonstrated that 7 of 38 (18.4%) WD patients had 24-h urinary Cu levels of <40 mcg at the time of diagnosis of WD, and their ages ranged from 1.3–8 years (median = 3 years), and 31.5% of WD children had 24-h urinary Cu levels <100 mcg at diagnosis of WD. In the same study, 4 of 58 (6.8%) patients, who were screened for WD due to an affected sibling or family member and/or elevated aminotransferases, had 24-h urinary Cu levels >40 mcg, and all were <100 mcg (13). By ROC analysis, a threshold of 40 and 100 mcg of 24-h urinary Cu level was reported to provide a diagnostic accuracy in identifying WD with 78.9% and 65.8% sensitivity, respectively (13). In contrast, a higher sensitivity of 86.4% was observed in our study with a threshold value of >40 mcg for the 24-h urinary Cu level. Nicastro et al. (13) also reported that the sensitivity for 24-h urinary Cu level at the cut-off value of 63.5 mcg was 95% and 70% in symptomatic and asymptomatic WD children, respectively. Another study by Sezer et al. demonstrated that decreasing the 24-h urinary Cu level threshold from 100 mcg to 67.5 mcg increased the sensitivity from 77% to 85% to identify WD in children; however, the specificity decreased from 76% to 71% (11). In the same study, 11.4% of WD patients' basal 24-h urinary Cu levels were <67.5 mcg, and 29.3% of non-WD patients' basal 24-h urinary Cu levels were >67.5 mcg (11). In another pediatric study, reducing the 24-h urinary Cu level threshold from 100 mcg to 52 mcg increased the sensitivity from 50% to 84.6% and the negative predictive value from 83.5% to 93.9% to identify WD, respectively; however, the specificity decreased from 97.1% to 91.2% (8). In our study, a 24-h urinary Cu level of >70 mcg was associated with high sensitivity (81.82%) for the diagnosis of WD in children. Furthermore, a 24-h urinary Cu level of >70 mcg/dl plus a serum ceruloplasmin level of <20 mg/dl exhibited the highest sensitivity and the highest positive and negative predictive values for the diagnosis of WD in children. The specificity of this combination was similar to that

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of a 24-h urinary Cu level of >100 mcg/dl plus a serum ceruloplasmin of <20 mg/dl.

Walshe reported that the basal 24-h urinary Cu excretion in hepatic WD (age range: 5-30 years) was more than twice that seen in presymptomatic WD (age range: 3-27 years) and more than 1.5 times that seen in neurological WD (age range: 7-39 years) (15). The basal 24-h urinary Cu level in patients with acute liver failure was found to be higher than in acute-on-chronic liver failure in WD (16). In the study by Sezer et al. (11), patients with cryptogenic cirrhosis and chronic hepatitis were included in the non-WD group, and 24-h urinary Cu levels were significantly higher in the cholestatic subgroup of the non-WD group. However, cholestasis had no effect on the 24-h urinary Cu levels in the WD group (14). In our study, the diagnostic cut-off value of 24-h urinary Cu level decreased to 55 mcg/dl after exclusion of the cholestatic patients. For threshold values of 100 mcg and 40 mcg for the 24-h urinary Cu level, there was no change in sensitivity after excluding cholestatic patients. In the literature, 24-h urinary Cu levels after penicillamine challenge has been a valuable test for the diagnosis of WD, notably to exclude WD (17). However, Nicastro et al. demonstrated that 24-h urinary Cu levels after penicillamine did not differ significantly between WD and non-WD patients ($p = 0.69$) (13). Additionally, it was suggested in the above mentioned study that Cu excretion was influenced by the severity of the liver damage because a positive penicillamine challenge test was observed in patients with histologically more severe liver damage in both the WD and non-WD groups (12).

In children, because it is difficult to collect an accurate 24-h urine and to assess the completeness of the urine collection and the accuracy of the urinary Cu excretion, creatinine excretion should be measured at the same time the 24-h urinary Cu levels are measured. In patients with celiac disease, 24-h urinary Cu levels were significantly higher than those of the controls (18). Additionally, urinary Cu excretion may be excessive in patients with renal tubular acidosis and WD. The limitations of our study were the lack of creatinine excretion measurement concurrently with the measurement of 24-h urinary Cu levels and the absence of screening for celiac disease and renal tubular acidosis.

In conclusion, the cut-off values of urinary Cu excretion for the diagnosis of WD in adults are not applicable to children. The diagnostic value of 24-h urinary Cu levels of >100 mcg/dl and >40 mcg/dl in children is not as sensitive and specific as in adult patients; therefore, the cut-off level of 24-h urinary copper in children has not been unequivocally established. We demonstrated that a 24-h urine Cu level >70 mcg/dl plus a ceruloplasmin level of <20 mg/dl and, after exclusion of the cholestatic patients, a 24-h urinary Cu level of >55 mcg/dl plus a serum ceruloplasmin level of <20 mg/dl are indicative of WD in children. However, while 24 h urinary Cu excretion is not sufficient as the sole diagnostic criterion for WD, it remains one of several clinical criteria leading to the diagnosis of WD. Thus, 24-h urinary Cu excretion and serum ceruloplasmin with liver function tests should be included in the diagnostic evaluation of suspected WD (19).

Conflict of interest

We declare that none of the authors have a financial interest related to this study. This study is not sponsored. Each author listed on the manuscript has read and approved the submission and takes full responsibility for the manuscript.

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