Pediatr Gastroenterol Hepatol Nutr. 2024 Jan;27(1):53-61
https://doi.org/10.5223/pghn.2024.27.1.53
pISSN 2234-8646·eISSN 2234-8840

Pediatric Gastroenterology, Hepatology & Nutrition
pghn

Original Article

Check for updates

Could Urinary Copper/Zinc Ratio Be a Newer Tool to Replace 24-Hour Urinary Copper Excretion for Diagnosing Wilson Disease in Children?

Fahmida Begum [1], **Khan Lamia Nahid** [1], **Tahmina Jesmin** [2], **Md. Wahiduzzaman Mazumder** [1] and **Md. Rukunuzzaman** [1]

[1]Department of Paediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh
[2]Department of Paediatric Nephrology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh

OPEN ACCESS

Received: May 10, 2023
Revised: Jul 6, 2023
Revised: Sep 5, 2023
Accepted: Oct 25, 2023
Published online: Jan 9, 2024

Correspondence to
Fahmida Begum
Department of Paediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka 1000, Bangladesh.
Email: fahmidalily@gmail.com

ORCID iDs
Fahmida Begum
https://orcid.org/0000-0003-3999-3428
Khan Lamia Nahid
https://orcid.org/0000-0002-0832-550X
Tahmina Jesmin
https://orcid.org/0000-0003-2787-3103
Md. Wahiduzzaman Mazumder
https://orcid.org/0000-0001-6947-9572
Md. Rukunuzzaman
https://orcid.org/0000-0003-0330-5080

ABSTRACT

Purpose: Although the 24-hours urinary copper excretion is useful for the diagnosis of Wilson disease (WD), there are practical difficulties in the accurate and timed collection of urine samples. The purpose of this study was to verify if the spot morning urinary Copper/Zinc (Cu/Zn) ratio could be used as a replacement parameter of 24-hours urinary copper excretion in the diagnosis of WD.

Methods: A cross-sectional study was conducted at the Department of Pediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, from June 2019 to May 2021 on 67 children over three years of age who presented with liver disease. Twenty-seven children who fulfilled the inclusion criteria for WD were categorized into the test group, and the remaining forty children were considered to have non-Wilsonian liver disease and were categorized into the control group. Along with other laboratory investigations, spot morning urinary samples were estimated for the urinary Cu/Zn ratio in all patients and were compared to the 24-hour urinary copper excretion. The diagnostic value of the Cu/Zn ratio was then analyzed.

Results: Correlation of spot morning urinary Cu/Zn ratio with 24-hours urinary copper excretion was found to be significant (r=0.60). The area under ROC curve with 95% confidence interval of morning urinary Cu/Zn ratio measured using 24-hours urine sample was 0.84 (standard error, 0.05; p<0.001).

Conclusion: Spot morning urinary Cu/Zn ratio seems to be a promising parameter for the replacement of 24-hours urinary copper excretion in the diagnosis of WD.

Keywords: Wilson disease; Children; Cu/Zn ratio; Spot urinary zinc; Spot urinary copper; Bangladesh

INTRODUCTION

Wilson disease (WD) is a rare autosomal recessive disease that affects approximately one in every 30,000 live births, with a higher prevalence in countries where consanguineous marriages are common [1]. In WD, a mutation in the *ATP7B* gene leads to reduced

Funding

This work was supported by Bangabandhu Sheikh Mujib Medical University (BSMMU) grant commission.

Conflict of Interest

The authors have no financial conflicts of interest.

ceruloplasmin formation and the excessive accumulation of copper in various organs, primarily affecting the liver, followed by the brain, cornea, and other organs. Copper accumulation typically occurs over an extended period after birth, with symptoms manifesting subsequently [2,3]. The prognosis for WD depends on early diagnosis and the prompt initiation of treatment. Remarkable hepatic damage occurs after the first decade, and neurological symptoms typically emerge after adolescence. Timely diagnosis, especially before the age of 10, can prevent significant organ damage through appropriate therapy [4-6]. Early intervention allows patients to enjoy an uneventful life like a healthy individual.

The diagnosis of WD in children poses a challenge due to the absence of a singular diagnostic test, aside from molecular genetic testing, which can definitively confirm or exclude WD. The advancement of molecular diagnostic techniques has significantly changed the natural course of WD, enabling precise diagnoses for both affected individuals and those with heterozygous genes through mutation analysis.

However, molecular genetic testing is an expensive and time-consuming procedure that has limited accessibility, particularly in resource-constrained countries. Additionally, there are over 600 mutations and polymorphisms associated with the *ATP7B* gene in WD, adding to the difficulty of the analysis. Alternatively, hepatic copper evaluation holds significant value as a diagnostic method for WD. Nonetheless, its invasive nature of sampling, potential for false-negative results, and inhomogeneous liver copper distribution preclude its effectiveness [7].

Although a combination of clinical features and laboratory parameters, such as Kayser-Fleischer (KF) rings, low serum ceruloplasmin concentrations, and increased urinary copper excretion, is commonly used to establish a diagnosis, none of these are pathognomonic for WD. KF rings, for instance, may not always be present in WD and can also occur in other chronic liver diseases (CLDs) [7,8]. Similarly, serum ceruloplasmin concentrations may appear normal in WD, while low concentrations can be observed in conditions such as protein-energy malnutrition, protein-losing enteropathy, or severely impaired hepatic function [4,9].

Among the diagnostic tests available, the 24-hour urinary copper (Cu) level is the simplest and most sensitive. This test reflects the amount of non-ceruloplasmin-bound Cu in circulation [10,11]. However, it requires precisely timed urinary sample collection, posing a practical challenge, especially in young children who may experience difficulties in providing complete and accurately timed urine samples. This may result in an inaccurate estimation of 24-hours urinary Cu excretion. Furthermore, the extended collection process introduces the risk of unexpected contaminations, and preserving a 24-hours urine sample can be challenging, particularly in hot weather.

Early diagnosis and treatment are the key determinants of prognosis; thus, it is important to develop a rapid, reliable, noninvasive, and discriminative diagnostic tool for WD. Herein, we investigated the copper/zinc (Cu/Zn) ratio as a potential alternative to the 24-hours urinary Cu excretion tool. Zinc was selected for this study because, like copper, zinc is a trace element, and both can be simultaneously detected using the same instrument [12,13].

In WD, there is an increase in copper accumulation in the liver, and urinary zinc excretion is highly correlated with hepatic copper concentration. Notably, we did not include iron despite being a trace element, as its urinary excretion does not correlate with hepatic copper

Pediatric Gastroenterology, Hepatology & Nutrition pghn

concentration [14]. Moreover, the Cu/Zn ratio has a positive association with hepatic copper concentration [14]. A previous study identified the urinary Cu/Zn ratio as a viable alternative to 24-hours urinary Cu excretion after penicillamine challenge in diagnosing WD [15]. Give the challenges associated with 24-hours urine collection in the pediatric population, our research used spot samples for both copper and zinc. The rationale for selecting copper and zinc is due to their properties of low diurnal variation for copper and intermediate variation for zinc [16]. Moreover, this approach is rapid, cost-effective, noninvasive, and straightforward, making it particularly suitable for countries with limited research resources.

MATERIALS AND METHODS

This cross-sectional study was conducted between June 2019 and May 2021 at the Paediatric Gastroenterology and Nutrition Department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, a tertiary-care hospital. The study protocol received approval from the local Ethics Committee at BSMMU (approval no. 12235). Informed written consent was obtained from each patient or their parents prior to participating in this study, and a guarantee of data privacy was provided. Additionally, parents and, where appropriate, the participants, were pre-trained for the accurate collection of urinary samples. Exclusion criteria encompassed individuals with abnormal renal functions, those who had undergone zinc therapy for any reason within 1 month of hospitalization, and those who did not adhere to the 24-hours urine collection instructions. No special dietary requirements were needed for the study purposes. None of the participants were using copper containers in their daily lives.

A total of 67 patients (ages 3–18 years) were enrolled in the study. Among them, 27 were diagnosed with WD and 40 with non-Wilsonian liver disease (non-WD), all of whom were hospitalized for liver-related conditions between June 2019 and May 2021. The diagnosis of WD was established with a score ≥4 according to the modified Leipzig score in children above 3 years of age [17]. The scoring system considered the following criteria:

1. Positive family history
2. Low serum ceruloplasmin (<20 mg/dL)
3. Elevated baseline 24-hours urinary Cu excretion (>100 μg/24 hr)
4. Presence of KF rings
5. Coombs' negative hemolytic anemia
6. Neurobehavioral symptoms

In addition to CLD, acute viral hepatitis was included as a control. Studies have indicated that both acute and chronic viral hepatitis can lead to an increase in urine copper excretion [18,19]. The justification for using acute viral hepatitis as a control lies in the observed rise in urine copper excretion associated with this condition.

Urinary sample collection and determination

We examined the serum ceruloplasmin levels and spot urinary Cu. The spot urinary Cu levels were then used to estimate the 24-hours urinary Cu levels. An eye examination was performed to identify the presence of KF rings and sunflower cataracts in all the participants. The diagnosis of WD was made according to the modified Leipzig scoring system. Unfortunately, we were unable to conduct urinary creatinine testing due to the unavailability of testing facilities at that time. Additionally, hepatic copper estimation and mutation analysis were not performed in our patients.

Pediatric Gastroenterology, Hepatology & Nutrition pghn

Urine samples were collected from all 67 patients (both WD and non-WD) in plastic bottles over a 24-hours period starting from 8.00 am. The total volume of the 24-hours urine collection was determined. For the estimation of both spot copper and zinc, two urinary samples (5 mL each) were collected from the last void urine using metal-free dispensable plastic tubes supplied by the atomic energy center. The 24-hours urinary Cu estimation was calculated by multiplying the spot urinary Cu result by the total volume of 24-hours urine. Due to various reasons, including unavailability and high-cost at the time of the study period, an acid-washed 24-hours urine container was not used. Because of the low diurnal variation of copper, we estimated the 24-hours urine Cu in this approach [16]. Additionally, some researchers have not identified diurnal variation in urine copper excretion in their studies, supporting the validity of estimating 24-hours urinary Cu excretion from spot morning urine Cu levels for accurate diagnosis [20]. These considerations served as the justification for our chosen approach in estimating 24-hours urinary Cu excretion.

A level of estimated copper excretion greater than 100 μg/24 hr was regarded as one of the parameters in the comprehensive assessment for the diagnosis of WD.

Copper and zinc levels in urine samples were determined simultaneously through flame atomic absorption spectrophotometry (FAAS). The 24-hours urinary Cu excretion was expressed as μg/24 hr, with 40 μg/24 hr used as the normal upper limit. An excretion exceeding 100 μg/24 hr was considered a diagnostic parameter for WD. The Cu/Zn ratio was calculated by dividing the copper concentration (μg/mL) by the zinc concentration (μg/mL). Notably, serum copper and zinc were not measured in the same session. Serum copper levels were not assessed because they can vary widely from low, normal, or high in cases of WD [21].

Typically, children with moderate acute malnutrition often exhibit zinc deficiency [22]. In the current study, among the patients with WD, 9 (33.1%) had no malnutrition, 17 (62.9%) had mild malnutrition, and only one (3.7%) exhibited moderate malnutrition. This distribution justifies our decision to omit the serum zinc level test. Moreover, our choice aligns with the approach taken by other researchers in similar studies who did not include the assessment of serum zinc levels in their investigations [15]. Serum ceruloplasmin was measured through an automated analyzer, the Architect Plus ci8200.

Statistical analysis

Descriptive analysis was performed to examine the demographic and clinical characteristics of the study participants. For comparison of continuous variables between two groups, the *t*-test was used, while chi-square test was used for categorical variables. Correlation analysis was performed using the Pearson test. The ANOVA test was employed to compare means across multiple groups where one variable is independent. The diagnostic accuracy of the 24-hours urinary Cu level was assessed using receiver operating characteristic (ROC) curves. Sensitivity and specificity were calculated for all cut-off levels, with 95% confidence intervals (CI). All cut-off values were defined as the probability of a true positive (sensitivity) and a true negative (specificity). Statistical analysis was performed using the IBM SPSS Statistics ver. 26.0 software (IBM Co.). All statistical tests were two-tailed, and statistical significance was set at a p-value<0.05.

Pediatric Gastroenterology, Hepatology & Nutrition pghn

RESULTS

The study encompassed 67 children, aged 3–18 years, of whom 27 were confirmed to have WD (score ≥4 according to modified Leipzig criteria) and 40 were classified as non-WD. The non-WD group comprised of 21 cases of acute hepatitis and 19 cases of CLDs.

In the acute hepatitis subgroup, 16 patients tested positive for the hepatitis A virus, three were positive for both the hepatitis A and E viruses, and the remaining two cases had non-A, non-B hepatitis. CLDs within the non-WD group encompassed various conditions, including cryptogenic CLD (n=13), autoimmune hepatitis (n=2), chronic hepatitis B virus (HBV) infection (n=1), caroli disease (n=1), and glycogen storage disease (n=2).

The demographic features, laboratory findings, including serum ceruloplasmin levels, and ophthalmological features are presented in **Table 1**. Of the 67 patients, 27 were diagnosed with WD with a mean age of 9.24±3.19 years, while 40 were identified as non-WD patients with a mean age of 9.2±3.12 years. There was no significant differences in demographic characteristics between WD patients and those without WD, except for a more common occurrence of positive family history in WD patients (p=0.007). Alanine aminotransferase (ALT) was observed to be higher in non-WD patients compared to WD patients (p=0.046). This difference could be attributed to the higher incidence of acute hepatitis cases in the control group (non-WD group). In this study, ALT levels exceeding ten times the normal value (>400 U/L) were considered high ALT. Serum ceruloplasmin levels were significantly lower in the WD group compared to the non-WD group (p<0.001), and Coombs' negative hemolytic anemia was significantly more prevalent in the WD group (p<0.001). There were also significant differences in the positivity of KF rings and in the presence of coagulopathy (p<0.001). A prothrombin time of INR 2.2 was chosen as a significant indicator of severe coagulopathy [23].

The WD group exhibited a significantly higher baseline 24-hours urinary Cu excretion, and this difference was statistically significant p<0.001. In the sub-analysis, comparing WD, acute viral hepatitis, and CLD, a significant difference in urinary copper levels between the groups was observed (p=0.009). The post-hoc analysis revealed a significant difference between the WD groups with acute hepatitis and those with CLD, but no significant difference was found between acute hepatitis and CLD. In contrast, urinary zinc levels did not show any significant difference between the groups.

Table 1. Clinico-pathological characteristics of the studied children (n=67)

Clinical and biochemical parameters	Wilson disease (n=27)	Non-Wilsonian liver disease (n=40)	p-value
Age	9.24±3.19	9.20±3.12	0.874†
Parental consanguinity	06 (22.2)	05 (12.5)	0.292‡
Positive family history	09 (33.3)	03 (7.5)	0.007‡
Low serum ceruloplasmin	27 (100.0)	5 (12.5)	<0.001‡
Coombs' negative hemolytic anemia	13 (48.1)	1 (2.5)	<0.001‡
Kayser-Fleischer ring	14 (51.8)	0 (0.0)	<0.001‡
Alanine aminotransferase (>400 U/L)	2 (7.4)	12 (30.0)	0.046‡
Low albumin (<30 mg/dL)	19 (70.3)	13 (32.5)	0.003‡
Severe coagulopathy (>2.2)	11 (40.7)	4 (10.0)	0.001‡

Values are presented as mean±standard deviation or number (%).
*Low serum ceruloplasmin level (<20 mg/dL).
†Students t-test.
‡Chi-square test.

https://doi.org/10.5223/pghn.2024.27.1.53

Table 2. Biochemical parameters of the studied participants (n=67)

Biochemical parameters	Wilson disease (n=27)	Non-wilsonian liver disease (n=40)	Non-wilsonian liver disease (n=40)		*p*-value
			Acute hepatitis (n=21)	Chronic liver disease (n=19)	
Urinary Cu/Zn ratio	9.14±24.62	0.27±0.44	-	-	<0.001*
Estimated baseline 24-hr urinary Cu excretion	1,623.88±3,101.89	50.57±20.84	-	-	<0.001*
Urinary Cu excretion after PCT/24 hr	4,691±8,396 (1,600–12,000)	747±599 (68–1,700)	-	-	<0.001*
Estimated 24-hr urinary Cu excretion	1,623.88±3,101.89	-	47.61±21.52	53.00±20.45	<0.009†
Spot urinary zinc excretion	1.33±2.10	-	0.87±1.12	0.60±0.60	<0.237†

Values are presented as mean±standard deviation (range).
Cu: copper, Zn: zinc, PCT: penicillamine challenge test, -: not available.
*Student's *t*-test.
†ANOVA test.

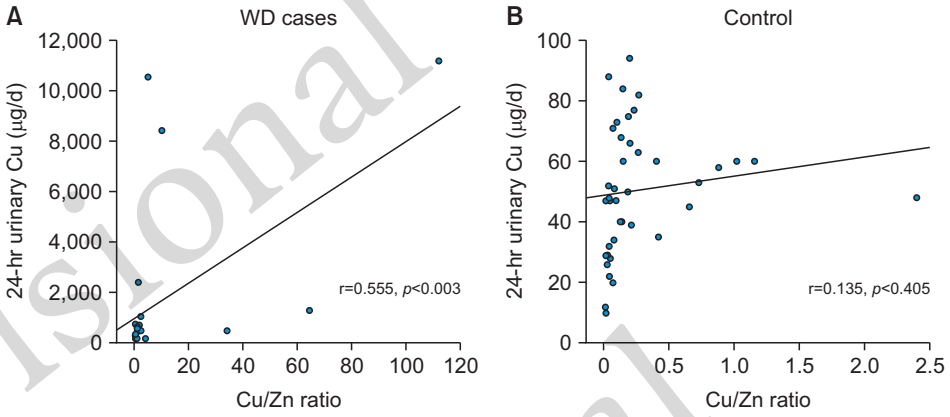

Fig. 1. (A) Correlation of spot urinary Cu/Zn ratio and 24-hours urinary Cu excretion for WD cases (n=27). (B) Correlation of spot urinary Cu/Zn ratio and 24-hours urinary Cu excretion for the control group (non-WD cases, n=40).
WD: Wilson disease, Cu: copper, Zn: zinc.

The spot urinary Cu/Zn ratio demonstrated a significant difference between the WD group and the non-WD group. The mean urinary zinc level for WD was 1.33 μg/mL, while the mean urinary zinc level for non-WD was 0.72 μg/mL. The mean Cu/Zn ratio for WD was 9.14±24.62 for the 27 cases, and 0.27±0.44 for the control group (**Table 2**). The Cu/Zn ratio, coupled with baseline 24-hours urine Cu in the 27 WD patients, showed a moderate but significant correlation (r=0.60, $p<0.001$) (**Fig. 1**).

The area under the ROC, with 95% CI, for the morning urinary Cu/Zn ratio measured using a 24-hours urine sample was 0.843 (95% CI: 0.743-0.940) (standard error, 0.05; $p<0.001$) (**Fig. 2**).

DISCUSSION

WD is one of the most common pediatric inborn metabolic disorders. Early diagnosis and treatment is crucial in possibly altering the natural course of the disease, leading to a more benign prognosis when identified at an early stage [24]. Typically, WD manifests in childhood with hepatic involvement. The presence of low ceruloplasmin levels and the KF ring can significantly facilitate the diagnosis of WD. However, diagnosing WD in children might be challenging, primarily due to the absence of the KF ring and the ambiguity surrounding serum ceruloplasmin levels. In certain WD patients, serum ceruloplasmin levels may be normal, while in some carriers, it may be low [24].

Pediatric Gastroenterology, Hepatology & Nutrition pghn

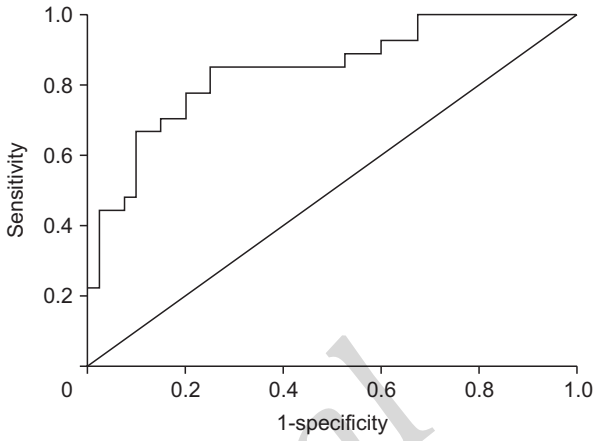

Fig. 2. Receiver operating characteristic curve for the spot urinary Cu/Zn ratio.
Cu: copper, Zn: zinc.

Therefore, numerous studies have emphasized the 24-hours urine Cu excretion as one of the most sensitive tests for diagnosing WD, and a test that can differentiate WD from other liver diseases in the pediatric population [25,26]. Despite the diagnostic value, the 24-hours urine collection method is time-consuming and challenging, particularly with the potential for incomplete collections that may yield unreliable results [27].

This prompted us to explore the feasibility of adopting a morning spot urine Cu/Zn ratio as an alternative to the 24-hours urinary Cu excretion. Copper and zinc are both trace elements that can be concurrently measured using the same equipment and may interact negatively with one another [12,13]. WD is characterized by increased hepatic copper accumulation, and urinary zinc excretion is highly correlated with hepatic copper concentration [14]. Due to significant hepatobiliary excretory impairment of copper, patients with WD may display a slightly elevated hepatic zinc concentration [28].

A positive association was observed between the morning urine Cu/Zn ratio and the estimated 24-hours urinary Cu excretion in patients with WD. This finding aligns with a similar positive correlation reported by Wang et al. [15]. Similar to the study, both the urine Cu/Zn ratios and baseline 24-hours urine Cu excretion demonstrated similar areas under the ROC curve. Notably, all participants with WD in this study were newly diagnosed and treatment-inexperienced. This study excluded individuals with a history of zinc and copper-containing multivitamin tablet use within a month of hospitalization.

To achieve accurate results, creatinine excretion measurement was performed simultaneously with the 24-hours urinary Cu excretion as it may be challenging to correctly collect urine from the younger patients. Furthermore, the presence of renal tubular acidosis in patients with WD could contribute to increased urine copper excretion. Unfortunately, a limitation of this study is the lack of concurrent creatinine excretion measurement and screening for renal tubular acidosis. Another limitation was the inability to store urine in an acid-washed container, necessitating the estimation of 24-hours urine Cu by multiplying the copper of the spot morning urine with the total 24-hours urinary volume.

A firm conclusion could not be formed from this study because of the relatively small sample size. However, the findings regarding the morning urine Cu/Zn ratio as a potentially

https://doi.org/10.5223/pghn.2024.27.1.53

promising alternative to the assessment of estimated 24-hours urinary Cu excretion for the diagnosis of WD are noteworthy. The simplicity and rapid collection of morning urine samples, on top of encouraging results, suggest the potential utility of this approach; however, a prospective trial involving a larger sample size is necessary.

REFERENCES

1. Hui J, Tang NLS, Li CK, Law LK, To KF, Yau P, et al. Inherited metabolic diseases in the Southern Chinese population: Spectrum of diseases and estimated incidence from recurrent mutations. Pathology 2014;46:375-82.
PUBMED | CROSSREF
2. Seo GH, Kim YM, Oh SH, Chung SJ, Choi IH, Kim GH, et al. Biochemical and molecular characterisation of neurological Wilson disease. J Med Genet 2018;55:587-93.
PUBMED | CROSSREF
3. Członkowska A, Litwin T, Dusek P, Ferenci P, Lutsenko S, Medici V, et al. Wilson disease. Nat Rev Dis Primers 2018;4:21.
PUBMED | CROSSREF
4. Riordan SM, Williams R. The Wilson's disease gene and phenotypic diversity. J Hepatol 2001;34:165-71.
PUBMED | CROSSREF
5. Roberts EA. Update on the diagnosis and management of Wilson disease. Curr Gastroenterol Rep 2018;20:56.
PUBMED | CROSSREF
6. Wiernicka A, Dądalski M, Jańczyk W, Kamińska D, Naorniakowska M, Hüsing-Kabar A, et al. Early onset of Wilson disease: Diagnostic challenges. J Pediatr Gastroenterol Nutr 2017;65:555-60.
PUBMED | CROSSREF
7. Piotr S, Wojciech J, Anil D, Ulrich B, Lorenzo D, Stuart T, et al. Wilson's disease in children: a position paper by the Hepatology Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. J Pediatr Gastroenterol Nutr 2018;66:334-44.
PUBMED | CROSSREF
8. Gow PJ, Smallwood RA, Angus PW, Smith AL, Wall AJ, Sewell RB. Diagnosis of Wilson's disease: an experience over three decades. Gut 2000;46:415-9.
PUBMED | CROSSREF
9. Mak CM, Lam CW. Diagnosis of Wilson's disease: a comprehensive review. Crit Rev Clin Lab Sci 2008;45:263-90.
PUBMED | CROSSREF
10. Yüce A, Koçak N, Demir H, Gürakan F, Ozen H, Saltik IN, et al. Evaluation of diagnostic parameters of Wilson's disease in childhood. Indian J Gastroenterol 2003;22:4-6.
PUBMED
11. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. Lancet 2007;369:397-408.
PUBMED | CROSSREF
12. Pang Y, Applegate TJ. Effects of dietary copper supplementation and copper source on digesta pH, calcium, zinc, and copper complex size in the gastrointestinal tract of the broiler chicken. Poult Sci 2007;86:531-7.
PUBMED | CROSSREF
13. Gao L, Ren S. Simultaneous spectrophotometric determination of four metals by two kinds of partial least squares methods. Spectrochim Acta A Mol Biomol Spectrosc 2005;61:3013-9.
PUBMED | CROSSREF
14. Fieten H, Hugen S, Van den Ingh TSGAM, Hendriks WH, Vernooij JCM, Bode P, et al. Urinary excretion of copper, zinc and iron with and without D-penicillamine administration in relation to hepatic copper concentration in dogs. Vet J 2013;197:468-73.
PUBMED | CROSSREF
15. Wang JS, Lu Y, Wang XH, Zhu QR. Urinary copper/zinc ratio: a promising parameter for replacement of 24-hour urinary copper excretion for diagnosis of Wilson's disease in children. World J Pediatr 2010;6:148-53.
PUBMED | CROSSREF

16. Moore RET, Rehkämper M, Kreissig K, Strekopytov S, Larner F. Determination of major and trace element variability in healthy human urine by ICP-QMS and specific gravity normalization. RSC Adv 2018;8:38022-35.
PUBMED | CROSSREF
17. Nagral A, Sarma SM, Matthai J, Kukkle PL, Devarbhavi H, Sinha S, et al. Wilson's disease: Clinical Practice Guidelines of the Indian National Association for Study of the Liver, the Indian Society of Pediatric Gastroenterology, Hepatology and Nutrition, and the Movement Disorders Society of India. J Clin Exp Hepatol 2019;9:74-98.
PUBMED | CROSSREF
18. Afridi HI, Kazi TG, Jamali MK, Sarfaraz RA, Arain MB, Kandhro GA, et al. Determination of copper and iron in biological samples of viral hepatitis (A-E) female patients. Biol Trace Elem Res 2009;129:78-87.
PUBMED | CROSSREF
19. Kalkan A, Bulut V, Avci S, Celik I, Bingol NK. Trace elements in viral hepatitis. J Trace Elem Med Biol 2002;16:227-30.
PUBMED | CROSSREF
20. Araki S, Murata K, Yokoyama K, Yanagihara S, Niinuma Y, Yamamoto R, et al. Circadian rhythms in the urinary excretion of metals and organic substances in "Healthy" men. Arch Environ Health 1983;38:360-6.
PUBMED | CROSSREF
21. Fernando M, Van Mourik I, Wassmer E, Kelly D. Wilson disease in children and adolescents. Arch Dis Child 2020;105:499-505.
PUBMED | CROSSREF
22. Kiio J, Ochola S, Nduati R, Kuria E, Mathenge S, Okoth J. Bioequivalence of micronutrient powders to Corn-soy Blend on serum zinc concentration of children (6-36 months) with Moderate Acute Malnutrition in Thika urban slums, Kenya: a cluster-randomized controlled trial. PLoS One 2022;17:e0274870.
PUBMED | CROSSREF
23. Alonso EM, Squires RH, Whitington PE. Acute Lliver Ffailure in Cchildren. In: Suchy FJ, Sokol RJ, Balistreri WF, editors. Liver Ddisease in Cchildren. Cambridge: 3rd ed. Cambridge University Press, 2007:71-96.
24. Roberts EA, Schilsky ML A practice guideline on Wilson disease. Hepatology 2003;37:1475-92.
PUBMED | CROSSREF
25. Schilsky ML. Non-invasive testing for Wilson disease: revisiting the d-penicillamine challenge test. J Hepatol 2007;47:172-3.
PUBMED | CROSSREF
26. Müller T, Koppikar S, Taylor RM, Carragher F, Schlenck B, Heinz-Erian P, et al. Re-evaluation of the penicillamine challenge test in the diagnosis of Wilson's disease in children. J Hepatol 2007;47:270-6.
PUBMED | CROSSREF
27. Morales JV, Weber R, Wagner MB, Barros EJ. Is morning urinary protein/creatinine ratio a reliable estimator of 24-hour proteinuria in patients with glomerulonephritis and different levels of renal function? J Nephrol 2004;17:666-72.
PUBMED
28. Stamoulis I, Kouraklis G, Theocharis S. Zinc and the liver: an active interaction. Dig Dis Sci 2007;52:1595-612.
PUBMED | CROSSREF