Diagnostic Accuracy of Serum Ceruloplasmin in the Diagnosis of Pediatric Wilson's Disease

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Abstract:

Background: *Wilson disease is a autosomal recessive disorder of copper metabolism with diverse clinical manifestation. Early diagnosis is crucial to prevent irreversible damage of liver & brain. Serum ceruloplasmin is one of the major diagnostic parameters for Wilson's disease. Herein, we evaluate diagnostic value of serum ceruloplasmin level for Wilson disease in children up to age 15 year.*

Materials & Methods: *This case control study was conducted from January 2016 to January 2019 in the department of Paediatric Gastroenterology and Nutrition, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh. Serum ceruloplasmin was measured in 103 WD patients and 58 non-WD patients with other liver disease. Wilson disease was diagnosed using the Leipzig score. Receiver operating characteristics (ROC) curve was used to determine the diagnostic accuracy of serum ceruloplasmin for WD in children.*

Results: *The mean age of WD patients was 9.97±2.5years, male female ratio was 1:1. The mean serum ceruloplasmin level in WD patient was7.76± 4.8, which was significantly lower than that in non-WD patients (34.29±11.17, p=0.00).The sensitivity, specificity, and accuracy of a ceruloplasmin level of <20 mg/dl in the discrimination of WD were 99%, 91%, and 96.2% respectively noted. The ROC curve revealed that serum ceruloplasmin level, at a cutoff value of 14.5 mg/ dl, had highest AUC value (0.992) with a sensitivity of 89.3% and specificity of 98.3%.*

Conclusion: *Serum ceruloplasmin is one of the sensitive biomarkers for the diagnosis of WD. The cutoff value of serum ceruloplasmin level at 14.5 may provide the highest accuracy for the diagnosis of WD.*

Key words: *Ceruloplasmin, Accuracy, Wilson's disease, Children.*

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Introduction:

Wilson's disease (WD) is a copper accumulation disorder caused by mutations in the Adenosine phosphatase 7B (ATP7B) gene[1]. It is an autosomal recessive inherited disease. Mutations in the ATP7B gene lead to failure of copper transport from hepatocyte into bile, so copper gets deposited in different organs, and the spectrum of clinical manifestations varies from progressive hepatic damage and neurological deterioration to atypical cardiac and osteoarticular involvement[2]. Timely diagnosis and early treatment are important to prevent permanent damage and to avert disease progression. Accurate and timely identification of WD is challenging due to protean clinical presentations. In 2003, a scoring system proposed by Ferenci et al.[3] for the diagnosis of WD (Leipzig score) that was based on a combination of clinical manifestations, laboratory tests, and genetic analysis.

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WD is easily recognized and diagnosed when patients present with typical symptoms using the Ferenci scoring system. However , for asymptomatic people and patient with unusual presentation, diagnosing WD is not easy. It is also unsuitable for screening purpose. Estimation of Liver copper in quantities >250 µg/g dry weight without cholestasis is considered to be the diagnostic gold standard but liver copper less than this threshold does not exclude the diagnosis[4].Furthermore, liver copper content may vary up to 500-fold and its distribution is unfavorably heterogenous[5]. Genetic analysis is the most decisive tool, but the test is expensive and time-consuming. Moreover, the significance of many mutations that occur in the ATP7B gene is undetermined[6]. Mutation analysis and liver copper estimation are not technically possible in Bangladesh.

Measurement of serum ceruloplasmin is the most widely accessible biochemical test for WD due to its rapidity and low cost. In addition, Further studies showed that low serum ceruloplasmin levels provided strong evidence for the diagnosis of WD, so the measurement of serum ceruloplasmin levels was recommended as the first step in screening for WD[6 7]. Serum ceruloplasmin levels below the low reference limit (conventionally taken as 0.20 g/L) is considered a diagnostic cutoff point for WD[1]. On the other hand, serum ceruloplasmin is age dependent, change with inflammation, may be low in cases of copper deficiency, nephrotic syndrome, chronic liver disease, protein-losing enteropathy, and hereditary aceruloplasminemia[8,99] Mak CM, Lam CW, Tam S. Diagnostic accuracy of serum ceruloplasmin in Wilson disease: determination of sensitivity and specificity by ROC curve analysis among ATP7B-genotyped subjects. Clinical chemistry. 2008 Aug 1;54(8):1356-62.

There have been few studies appraising the diagnostic accuracy of serum ceruloplasmin.in children.

The objective of this study was to assess the diagnostic value of ceruloplasmin for screening and early diagnosis of WD in children and to determine the optimal cutoff value of serum ceruloplasmin levels for the diagnosis of WD in Bangladesh.

Materials & Methods:

A total of 103 children with WD diagnosed at the Department of Pediatric Gastroenterology and Nutrition, BSMMU, Dhaka, Bangladesh from January 2016 through January 2019 were enrolled in this study. WD was diagnosed according to Leipzig score >4[1,10] without taking in account of mutation analysis and hepatic copper estimation. Liver disease Patients with other etiologies were selected as control. Patients with cholestasis, severe malnutrition or protein losing enteropathy and who were unwilling to give consent were excluded from the study.

Detailed history and clinical examinations including presence of jaundice, ascites, hepatosplenomegaly, sign of liver failure and neurological assessment were done by researcher herself. Slit-lamp eye examination was done in each patient at Ophthalmology Department of BSMMU by a single expert ophthalmologist and results were recorded in a data collection sheet. Hepatic copper estimation and mutation analysis cannot be done because of unavailability of the test in Bangladesh. Basic laboratory test like (CBC with PBF, reticulocyte count, prothrombin time, and coomb's test) were done at Department of Hematology of BSMMU. Serum ceruloplasmin were measured by Nephelometric assay using the Beckman Coulter Image. The serum level of albumin, ALT, bilirubin and serum total protein were done at the Department of Biochemistry, BSMMU. Additional tests were done as necessary to determine the causes of liver disease such as screening for HAV, HBV, HEV and autoimmune screening like ANA, SMA, LKM1.

Statistical analysis:

Statistical analyses were performed using SPSS (version 20; SPSS). Continuous quantitative variables (age, serum Ceruloplasmin) were expressed as the means and standard deviations (SD). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for assessment of diagnostic accuracy. The receiver operating characteristic (ROC) curve, which was used to determine the optimal cutoff value of serum ceruloplasmin for diagnosis of WD, was constructed using the data from WD patients and those with other various diseases. A value of $p < 0.05$ was considered statistically significant.

Results:

Study population comprised of 161 children including 103(63.9%) Wilson disease and 58(36.02%) non-Wilson disease. The mean age of WD patients was 9.97±2.5 years. Of the WD patients, 53 were female and 50 were male. Among non- WD patients 30 cryptogenic CLD, 2 chronic hepatis B , 11 acute hepatitis A, 7 acute liver failure due to hepatitis A, 4 autoimmune hepatitis, 1 caroli disease, 1 biliary cirrhosis, 2 glycogen storage disease. Regarding the various manifestations of Wilson's disease, 93.7 % of patients had only hepatic manifestations, in the form of CLD 62(60.1%), ALF 23 (22.3%), acute hepatitis 9(8.7%) and 7 (6.7%) patients also had neurological manifestation along with liver

disease. Two (1.9%) patients had asymptomatic transaminaesemia identified during family screening. The basic characteristics of the study populations are shown in Table I. The mean ceruloplasmin level in the 103 patients was 7.76± 4.8 mg/dl, which was significantly lower than that in the non-WD patients (P<0.00). Of the103 people with WD,86 had a KF ring. Family h/o of liver disease is more common in WD group which is statistically significant.

Gender Specific reference value of serum ceruloplasmin was analyzed among WD & non- WD group (Fig:1). Serum Ceruloplasmin level was higher in girls in WD group, but in non-WD serum ceruloplasmin lower in girls than boys. There is no significant difference.

Serum ceruloplasmin level is significantly lower in children with WD in comparison to non-WD group (p value=0.001) (Table II).

Diagnostic accuracy of serum ceruloplasmin in Wilson's disease

The ROC curve (Fig:2A) was constructed by using the data of 103 WD patients & non-WD patients with hepatic disease. As shown in Fig. 2A, the conventional cutoff value of 20 mg/dL gave a sensitivity of 99% and a specificity of 91%. The positive and negative predictive values were 95.28% and 98.14%, respectively. The ROC curve analysis indicated that the cutoff value of serum ceruloplasmin level of 14.5 mg/dL provided the highest AUC value of 0.992 (95% confidence interval (CI), 0.983–1.000), with a sensitivity of 89.3% and specificity of 98.3%. The positive and negative predictive values of serum ceruloplasmin level of 14.5 mg/dl were 98.9% and 83.8%, respectively.

As shown in Fig. 2B-D, the mean level of serum ceruloplasmin of 103 WD patients was 7.76 ± 4.7 mg/dL, significantly lower than that in all of 58 children (34.29± 11.17mg/dL) ($p < 0.001$). For the patients with acute liver failure, the mean level of serum ceruloplasmin in WD patients (8.06 ± 5.58 mg/dL) was lower than that in non-WD patients (34.43 ± 11.8 mg/dL) ($p < 0.001$). In the situation with elevated aminotransferases, the mean level of serum ceruloplasmin in the WD patients (8.06 ± 5.5 mg/dL) was significantly lower than that in the 38 patients with viral hepatitis (34.43± 11.8mg/dL) ($p < 0.001$).

Table-I

Basic Characteristics	WD group (n=103)	Non-WD (n=58)	P -value
Age (Y)	9.97±2.59	9.35±2.98	0.17
Male	50(48.5%)	36(62.06%)	0.09
S. ceruloplasmin	7.76± 4.8	34.29±11.17	0.00
KF ring	81	0	0.00
Family h/o of liver disease	26	4	0.004

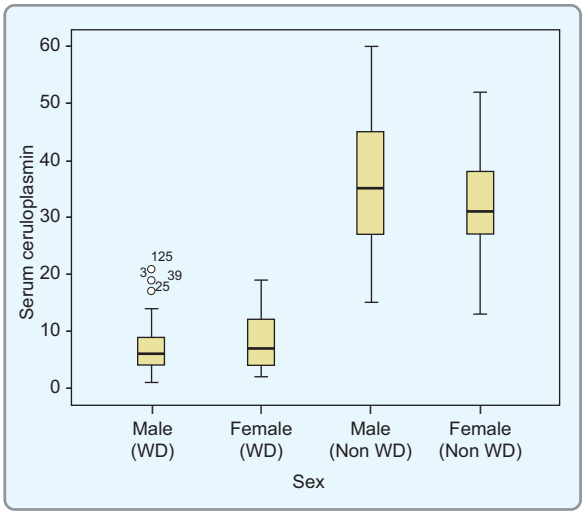


Figure-1: *Gender difference of serum ceruloplasmin was analyzed among WD and non WD group.*

Table-II

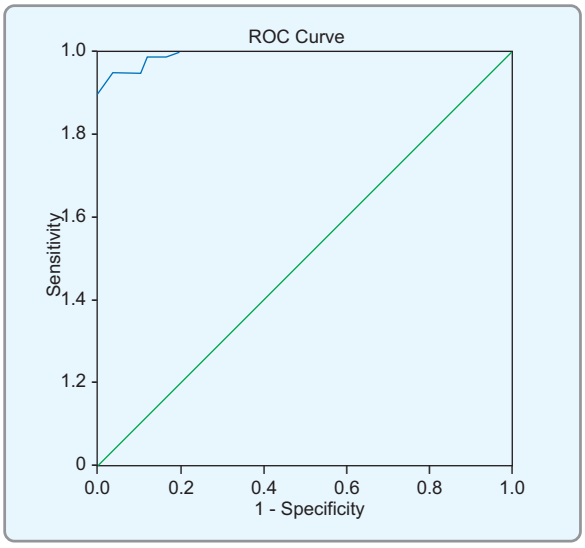
Distribution of serum ceruloplasmin level in WD from non -WD.

		Group diagnosis WD n(%)	NON-WD n(%)	Total	p-value
SERUM CERULOPLASMIN (MG/DL)	<10	71(68.9)		0(00.00)	71(44.09)
	10-20	31(30.09)		5(8.6)	35(21.73)
	>20	1(0.97)		54 (93.1)	55(34.16)
	TOTAL	103(100)		58(100)	161(100)

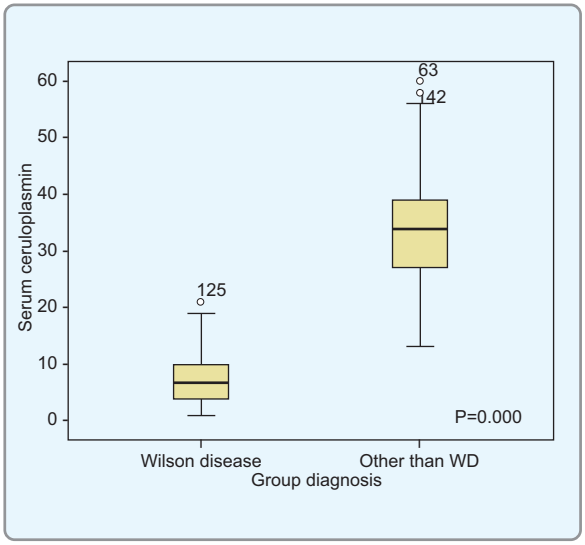
Table-III

Diagnostic accuracy of serum ceruloplasmin testing

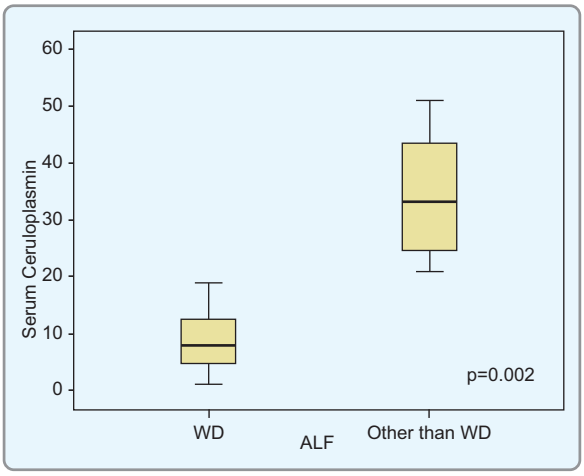
Cut -off level of ceruloplasmin (mg/dl)	WD group (n=103)	Non -WD (n=58)	Sensitivity(%)	Specificity(%)	Accuracy(%)
<20	102	5	99%	91%	96.2%
≥20	1	53			
<14.5	92	1	89.3%	98.3%	92.5%
≥14.5	11	57			



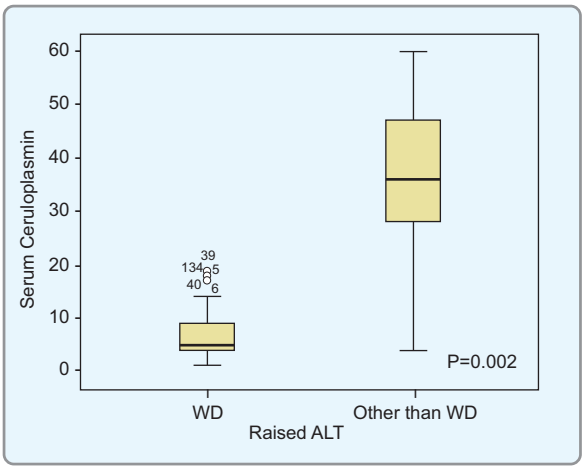
A. The ROC Curve for WD with conventional cut off of 20mg/dl



B. The Box and whisker plots for WD with conventional cut off of 20mg/dl



C. The Box and whisker plots for WD and non WD with ALF



D. The Box and whisker plots for WD and non WD with raised ALT

Discussion:

A ceruloplasmin concentration <20 mg/dl is conventionally considered as one of the major diagnostic tool for WD. But the lower reference varies with different analytical methods, ethnicity, the age of the reference subjects and clinical presentation. Therefore some studies have been conducted to find the cutoff value of ceruloplasmin for the diagnosis of wilson based on local population. Here, we recruited a small Bangladeshi cohort to determine the diagnostic accuracy of serum ceruloplasmin for diagnosis of Wilson's didease. Although diagnosis of WD must be based on both clinical and laboratory findings, measurement of ceruloplasmin is a rapid and inexpensive biomarker. However , there is not much pediatric data available on ceruloplasmin. The present study recruited 103 pediatric WD cases with 58 paeditric patients as control. In this study low ceruloplasmin level was found in 103(99%) cases that is close to the results reported by other authors [11,1212] Manolaki N, Nikolopoulou G, Daikos GL, Panagiotakaki E, Tzetis M, Roma E, Kanavakis E, Syriopoulou VP. Wilson disease in children: analysis of 57 cases. Journal of pediatric gastroenterology and nutrition. 2009 Jan 1;48(1):72-7.

Consistent with the reports from other studies from China and South Korea, our findings showed that low sreum ceruloplasmin is strongly reflecting the abnormality of copper metabolism and potential diagnosis of WD[13,14]. A sensitive biomarker for the diagnosis of WD could be serum ceruloplasmin as it is statistically significantly lower in WD patients than in the non-WD group.Previous study has shown that there are no sex related difference in ceruloplasmin[15,16]. Our study also consistant with this findings.

The most common metabolic causes of ALF in children include WD. In our study, we have 23 WALF and 7 Non wilson ALF. The wilsonian ALF was larger than non wilson ALF due to refferal bias.Patients with Wilson's disease may have received priority for admission due to severity of their disease.Other tertiary hospital can diagnose acute liver failure of viral origin and most of them can be managed conservatively.In this study showed that ceruloplasmin in acute liver failure with WD was significantly lower than other etiology of acute liver failure. This is consistent with two other studies[17,18] showing that serum ceruloplasmin measurement appears to be the fastest and most important test, particularly in an urgent life-threatening situation such as fulminant hepatitis.

In this study, serum ceruloplasmin concentration <20 mg/dl showed sensitivity, specificity, accuracy is 99%,91% and 96.2% respectively. Lu et al. [Error! Reference source not found.](found sensitivity, specificity of a ceruloplasmin level of <20mg/dl were 98.1% & 86.5% and 84.8 %, respectively. Here, using threshold of 20 mg/dl led to 5 false positive. Most of them due to cryptogenic CLD. Low concentrations of ceruloplasmin can be observed in non-WD patients with decompensated liver failure, whose synthetic function is severely impaired. An abnormal

ceruloplasmin level can lead to further investigations, patient anxiety and even lead to chelation treatment.Therefore, additional effort could be made to rule out WD and occasional early mis-treatment could be performed in the Non-WD children with low ceruloplasmin.Infact, there is no diagnostic modality to disgnose WD other than genetic test.

We derived an ROC curve of 103 bangladeshi children to investigate the optimal cutoff value of serum ceruloplasmin for the diagnosis of WD. It is controversial whether the optimal cutoff value of serum ceruloplasmin levels for the diagnosis of WD is between 11.5 mg/dL to 20 mg/dL[9,Error! Reference source not found.,19,20,21,22,23]. In the present study, the ROC curve analyses showed the optimal cutoff value of serum ceruloplasmin is 14.5 mg/dL, with a sensitivity of 89.3% and specificity of 98.3%, thereby providing higherst diagnostic accuracy for WD in children. These results were consistent with those of previous studies from China and Korea[14,20].

For a screening test of a very low-prevalence disease such as WD, a low threshold should be chosen to favor specificity. Using the 14.5 mg/dl threshold, the sensitivity only decreased by 10.7%, and the false-positive rate decreased by 6.8%, which in turn resulted in 4 false-positive patients prevented unnecessary further investigations. Interpretation of ceruloplasmin using a threshold of 14.5 mg/dl resulted in higher specificity and positive predictive value. Therefore, the new threshold may be more cost-effective than the conventional one. However, the sample size in this study is small. With regards to the introduction of a new cut off value in the measurement of serum ceruloplasmin, no study has been carried out to assess the cost, clinical and economic benefits. Therefore, further study is required with large sample size.

In conclusion , Serum ceruloplasmin is one of the sensitive biomarker for the diagnosis of WD. The cutoff value of serum ceruloplasmin level at 14.5 may provide highest accuracy for the diagnosis of WD. Study suggested that the diagnostic value of ceruloplasmin could be strengthened by adopting a new cut-off level to avoid false postivity. We, recommend that each laboratory establish the referance interval based on the local population. However, to adopt a lower cut-off value of serum ceruloplasmin as a first- line screening test, a cost - benefit analysis is required to overcome reduced sensitivity.

Conflict of interest: None

Reference:

1. European Association For The Study Of The Liver. EASL clinical practice guidelines: Wilson's disease. Journal of hepatology. 2012 Mar 1;56(3):671-85.
2. Dzie¿yc-Jaworska K, Litwin T, Cz³onkowska A. Clinical manifestations of Wilson disease in organs other than the liver and brain. Annals of Translational Medicine. 2019 Apr;7(Suppl 2).
3. Ferenci P, Caca K, Loudianos G, Mieli Vergani G, Tanner S, Sternlieb I, Schilsky M, Cox D, Berr F. Diagnosis and phenotypic classification of Wilson disease 1. Liver International. 2003 Jun;23(3):139-42.
4. Ferenci P, Steindl-Munda P, Vogel W, Jessner W, Gschwantler M, Stauber R, Datz C, Hackl F, Wrba F, Bauer P, Lorenz O. Diagnostic value of quantitative hepatic copper determination in patients with Wilson's Disease. Clinical Gastroenterology and Hepatology. 2005 Aug 1;3(8): 811-8.
5. McDonald JA, Snitch P, Painter D, Hensley W, Gallagher ND, McCaughan GW. Striking variability of hepatic copper levels in fulminant hepatic failure. Journal of gastroenterology and hepatology. 1992 Aug;7(4):396-8.
6. Roberts EA, Schilsky ML. Diagnosis and treatment of Wilson disease: an update. Hepatology. 2008 Jun;47(6):2089-111.
7. Yang Y, Hao W, Wei T, Tang L, Qian N, Yang Y, Xi H, Zhang S, Yang W. Role of serum ceruloplasmin in the diagnosis of Wilson's disease: A large Chinese study. Frontiers in Neurology. 2022 Dec 7;13:1058642.
8. Hellman NE, Gitlin JD. Ceruloplasmin metabolism and function. Annual review of nutrition. 2002 Jul;22(1): 439-58.
9. Mak CM, Lam CW, Tam S. Diagnostic accuracy of serum ceruloplasmin in Wilson disease: determination of sensitivity and specificity by ROC curve analysis among ATP7B-genotyped subjects. Clinical chemistry. 2008 Aug 1;54(8):1356-62.
10. Ferenci P, Caca K, Loudianos G, Mieli Vergani G, Tanner S, Sternlieb I, Schilsky M, Cox D, Berr F. Diagnosis and phenotypic classification of Wilson disease 1. Liver International. 2003 Jun;23(3):139-42.
11. Taly AB, Meenakshi-Sundaram S, Sinha S, Swamy HS, Arunodaya GR. Wilson disease: description of 282 patients evaluated over 3 decades. Medicine. 2007 Mar 1;86(2): 112-21.
12. Manolaki N, Nikolopoulou G, Daikos GL, Panagiotakaki E, Tzetis M, Roma E, Kanavakis E, Syriopoulou VP. Wilson disease in children: analysis of 57 cases. Journal of pediatric gastroenterology and nutrition. 2009 Jan 1;48(1):72-7.

13. Lu X, Li S, Zhang W, Lin Y, Lu Z, Cai Y, Su X, Shao Y, Liu Z, Sheng H, Huang Y. Assessment of the diagnostic value of serum ceruloplasmin for Wilson's disease in children. BMC gastroenterology. 2022 Mar 16;22(1):124.

14. Xu R, Jiang YF, Zhang YH, Yang X. The optimal threshold of serum ceruloplasmin in the diagnosis of Wilson's disease: A large hospital-based study. PLoS One. 2018 Jan 11;13(1):e0190887.

15. Clifford SM, Bunker AM, Jacobsen JR, Roberts WL. Age and gender specific pediatric reference intervals for aldolase, amylase, ceruloplasmin, creatine kinase, pancreatic amylase, prealbumin, and uric acid. Clinica Chimica Acta. 2011 Apr 11;412(9-10):788-90.

16. Schonfeld EA, Brown RS. Genetic testing in liver disease: what to order, in whom, and when. Clinics in Liver Disease. 2017 Nov 1;21(4):673-86.

17. Korman JD, Volenberg I, Balko J, Webster J, Schiodt FV, Squires Jr RH, Fontana RJ, Lee WM, Schilsky ML, Pediatric and Adult Acute Liver Failure Study Groups. Screening for Wilson disease in acute liver failure: a comparison of currently available diagnostic tests. Hepatology. 2008 Oct;48(4):1167-74.

18. Yasmin A, Rukunuzzaman M, Karim AB, Alam R, Hossen K, Sonia ZF. Ratio of aspartate aminotransferase to alanine aminotransferase and alkaline phosphatase to total bilirubin in Wilsonian acute liver failure in children. Indian Journal of Gastroenterology. 2022 Jun;41(3):224-30.

19. Yang Y, Hao W, Wei T, Tang L, Qian N, Yang Y, Xi H, Zhang S, Yang W. Role of serum ceruloplasmin in the diagnosis of Wilson's disease: A large Chinese study. Front Neurol. 2022 Dec 7;13:1058642.

20. Kim JA, Kim HJ, Cho JM, Oh SH, Lee BH, Kim GH, Choi JH, Kim KM, Yoo HW. Diagnostic value of ceruloplasmin in the diagnosis of pediatric Wilson's disease. Pediatric Gastroenterology, Hepatology & Nutrition. 2015 Sep 1;18(3):187-92.

21. Nicastro E, Ranucci G, Vajro P, Vegnente A, Iorio R. Re evaluation of the diagnostic criteria for Wilson disease in children with mild liver disease. Hepatology. 2010 Dec;52(6):1948-56.

22. Mohr I, Weiss KH. Biochemical markers for the diagnosis and monitoring of Wilson disease. The Clinical Biochemist Reviews. 2019 May;40(2):59.

23. García-Villarreal L, Hernández-Ortega A, Sánchez-Monteagudo A, Peña-Quintana L, Ramírez-Lorenzo T, Riaño M, Moreno-Pérez R, Monescillo A, González-Santana D, Quiñones I, Sánchez-Villegas A. Wilson disease: revision of diagnostic criteria in a clinical series with great genetic homogeneity. Journal of Gastroenterology. 2021 Jan;56:78-89.