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Is it possible to diagnose fulminant Wilson's disease with simple laboratory tests?

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Abstract

Background: Wilson's disease is a rare cause of acute liver failure and is highly fatal without liver transplantation. Fast and accurate diagnostic methods are needed for fulminant Wilson's disease (FWD). In this study, we aimed to develop an early, simple and accurate diagnostic method to differentiate FWD from nonwilsonian acute liver failure (NWALF) causes using routine biochemical data.

Methods: The medical records of 24 paediatric FWD and 120 paediatric NWALF cases diagnosed at the Department of Pediatric Gastroenterology, Hepatology, and Nutrition between January 2007 and February 2017 were retrospectively reviewed.

Results: Using receiver operator characteristics curve (ROC) analysis, we have determined the best cut-off point for laboratory findings in FWD. Patients meeting these cut-off points were assigned one point and others were assigned zero point. We then formed a new variable consisting of the combination of 14 variables and performed a new ROC analysis. We obtained a cut-off point of ≥4.5 for FWD. The diagnostic performance of the score was characterized by a sensitivity of 0.889, a specificity of 0.879 ($P < .001$). A scoring system based only on aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, AST/ALT ratio, uric acid and haemoglobin had a best cut-off point of ≥2.5 for FWD, which had a sensitivity of 0.875, a specificity of 0.867 ($P < .001$).

Conclusions: Our study demonstrated that biochemical markers offer almost as reliable, fast and accurate diagnosis of FWD as offered by ceruloplasmin and 24-hour urinary copper.

KEYWORDS

children, early diagnosis, fulminant Wilson's disease, simple biochemical markers

1 | INTRODUCTION

Wilson's disease (WD) is an uncommon cause of acute liver failure and is highly fatal without liver transplantation (LT).[1] However, it is almost impossible to obtain diagnostic marker results including ceruloplasmin, 24-hour urinary copper and hepatic copper content within a short and critical period in those patients.[2] Thus, it seems that rapid and accurate diagnostic methods are needed for fulminant Wilson's disease (FWD). In this study, we aimed to develop an early, simple and accurate diagnostic method for differentiation of FWD from other causes of nonwilsonian acute liver failure (NWALF) using routine biochemical data.

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; D.bil, direct bilirubin; FWD, fulminant Wilson's disease; GGT, gammaglutamyl transferase; Hb, Haemoglobin; LT, liver transplantation; NWALF, nonwilsonian acute liver failure; T.bil, total bilirubin; WD, Wilson's disease.

2 | MATERIALS AND METHODS

The medical records of 24 paediatric FWD and 120 paediatric NWALF cases diagnosed at the Department of Pediatric Gastroenterology, Hepatology, and Nutrition between January 2007 and February 2017 were retrospectively reviewed.

Acute liver failure was defined in accordance with Pediatric Acute Liver Failure Study Group criteria[1,3,4]: (a) children with no evidence of chronic liver disease, (b) biochemical evidence of acute liver injury, (c) hepatic-based coagulopathy (defined as prothrombin time [PT] ≥15 seconds or International Normalized Ratio (INR) ≥1.5 not corrected by vitamin K in the presence of hepatic encephalopathy; or PT ≥20 seconds or INR ≥2.0 regardless of the presence or absence of clinical encephalopathy).

In patients who scored 4 or more according to the scoring system developed at the International Wilson Meeting in Leipzig in 2001, these patients were considered FWD if they had findings of acute liver failure according to the Pediatric Acute Liver Failure Working Group.[3,5-7]

All FWD patients had ceruloplasmin <0.1 g/L and 24-hour urinary copper levels >140 μgr/24 h. In 12 of the 14 patients who underwent LT, copper levels were measured in the explant liver. In 6 of the 10 patients who did not undergo transplantation, the level of liver copper was observed after the coagulation disorder improved. In 6 patients with no hepatic copper level, there was a Kayser-Fleischer (KF) ring and Coombs negative haemolytic anaemia.

Other NWALF group: A specific cause of NWALF could only be identified in 48 (40%) patients. Toxic liver injury was found in 30 (25%) patients; drug-induced NWALF in 11, poisoning due to yellow phosphorus in 8, amanita in 6, narcotics in 3 and plants in 2 children. Nine children (7.5%) had infectious causes (hepatitis A in 7 and hepatitis B in 2 patients), 4 (3.4%) had autoimmune hepatitis, 3 (2.5%) had secondary haemophagocytic lymphohistiocytosis and 2 (1.6%) had Budd-Chiari syndrome. The cause could not be determined in 72 children (60%).

Biochemical data were obtained from the first samples taken immediately after admission.

The local ethics committee approved the study protocol.

Data were statistically analysed with Statistical Package for the Social Sciences for Windows (SPSS Inc) 22.0. The continuous variables were reported as the mean ± standard deviation, whereas the categorical variables were defined as percentages. The data were tested for normal distribution using the Kolmogorov-Smirnov test. To compare the continuous variables, Student's *t* test, a one-way analysis of variance test or a Kruskal-Wallis test was used, as appropriate.

The cut-off points for FWD for each measurement were calculated by ROC analysis. Then all the variables were coded as 1 and 0, referring to FWD and NWALF respectively. We generated a new variable consisting summation of the 14 and 6 coded variables and then applied a new ROC analysis.

Key points

- The results of diagnostic markers such as ceruloplasmin, 24-hour urinary copper and hepatic copper content are difficult to obtain in patients with fulminant Wilson's disease (FWD).
- Fast and accurate diagnostic methods are needed for FWD. Our study showed that in paediatric patients presenting with acute hepatic impairment, simple biochemical markers provide reliable, rapid and accurate diagnosis of FWD.
- It will allow the treatment regimens for FWD to start faster.

3 | RESULTS

An analysis based upon demographic variables showed that the mean age of the FWD and NWALF groups was 8.65 ± 2.80 (4-13) years, and 5.98 ± 4.68 (0.5-17) years respectively ($P < .001$). An analysis of gender distribution showed that both groups were similar ($P = .823$).

Ceruloplasmin level was measured in 43 NWALF patients and all FWD patients. Mean levels were 11.04 ± 4.78 mg/dL and 24.86 ± 7.81 mg/dL in FWD and NWALF groups respectively ($P < .001$). An ROC analysis showed that the best cut-off point ≤19.5 for FWD had an AUC of 0.932(0.868-0.995) ($P < .001$), a sensitivity of 0.814, and a specificity of 1.

Twenty-four-hour urinary copper was measured in 50 patients with NWALF and all patients with FWD. Mean urinary copper was 1691.58 ± 1589.98 μg/24 h and 118.95 ± 123.58 μg/24 h in FWD and NWALF groups respectively ($P < .001$). An ROC analysis showed that the best cut-off point of ≥239.5 μg/24 h for FWD had an AUC of 0.965 (0.930-1) ($P < .001$), a sensitivity of 0.917, and a specificity of 1.

Twenty-four-hour urinary copper content with D-penicillamine was measured in 21 NWALF patients and 8 FWD patients. The mean levels were 3274.50 ± 3938.47 μg/24 h and 361.93 ± 185.09 μg/24 h in FWD and NWALF groups respectively ($P = .002$). An ROC analysis revealed that the best cut-off point of ≥747.5 μg/24 h for FWD had an AUC of 0.949 (0.850-1) ($P < .001$), a sensitivity of 0.875, and a specificity of 1.

An analysis based upon laboratory findings revealed that serum levels of total bilirubin (T.bil), direct bilirubin (D.bil), gammaglutamyl transferase (GGT), and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio were significantly higher in FWD than in NWALF ($P = .001$, $P = .006$, $P = .005$ and $P < .001$ respectively). Haemoglobin (Hb), platelet (PLT), ammonia, albumin, cholesterol, AST, ALT, alkaline phosphatase (ALP), uric acid and ALP/T.bil ratio values were significantly lower in FWD compared to NWALF ($P < .001$, $P = .009$, $P < .001$, $P = .005$, $P < .001$, $P < .001$, $P < .001$, $P < .001$ and $P = .026$ respectively) (Table 1).

TABLE 1 Comparison of laboratory data of children with FWD or NWALF

	NWALF (120)	FWD[24]	*P*
WBC (10^3/mL)	10.99 ± 6.09	12.87 ± 7.16	.182
Hb (g/dL)	10.98 ± 2.29	8.55 ± 2.45	<.001
PLT (10^3/mL)	276.5 ± 155.63	185.37 ± 139.85	.009
INR	4.43 ± 2.52	4.8 ± 1.78	.502
Ammonia (μg/dL)	302.05 ± 281.65	187.5 ± 63.51	<.001
T.protein (mg/dL)	7.17 ± 12.18	5.61 ± 1.03	.532
Albumin (mg/dL)	3.19 ± 0.62	2.80 ± 0.56	.005
Triglycerides (mg/dL)	111.38 ± 59.01	98.40 ± 42.68	.366
Cholesterol (mg/dL)	107.39 ± 40.08	68.30 ± 36.56	<.001
LDL (mg/dL)	80.37 ± 63.22	50.16 ± 31.60	.055
HDL (mg/dL)	11.28 ± 8.28	9.35 ± 10.84	.402
T. bilirubin (mg/dL)	16.32 ± 9.39	23.80 ± 11.69	.001
D. bilirubin (mg/dL)	10.34 ± 6.51	14.63 ± 8.19	.006
AST (U/L)	2716.34 ± 3799.00	308.16 ± 298.90	.002
ALT (U/L)	2111.31 ± 2530.97	165.66 ± 272.60	<.001
ALP (U/L)	360.78 ± 187.92	161.29 ± 153.30	<.001
GGT (U/L)	56.30 ± 47.35	88.75 ± 64.72	.005
Uric acid (mg/dL)	4.03 ± 3.63	0.95 ± 0.36	<.001
Mg (mg/dL)	2.16 ± 0.41	1.99 ± 0.38	.060
AST/ALT	1.36 ± 0.78	4.26 ± 3.69	<.001
AST/ALP	9.31 ± 15.00	5.65 ± 9.89	.256
ALP/T.bil	38.18 ± 38.83	18.59 ± 38.92	.026

Abbreviations: NWALF, nonwilsonian acute liver failure, ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FWD, fulminant Wilson's disease; GGT, gammaglutamyl transferase; Hb, Haemoglobin; PLT, platelet; T.bil, total bilirubin.

TABLE 2 Determination of the best cut-off points of laboratory parameters for discrimination of FWD from NWALF due to other aetiologies

Variable	Cut-off value for FWD	Sensitivity	Specificity	AUC (95% CI)	*P* value for AUC
Hb (g/dL)	≤9.15	0.667	0.767	0.763 (0.648-0.877)	<.001
PLT (10^3/mL)	≤169.5	0.667	0.742	0.703 (0.577-0.829)	.002
Albumin (mg/dL)	≤2.95	0.778	0.638	0.698 (0.581-0.814)	.002
Cholesterol(mg/dL)	≤97.0	0.778	0.655	0.763 (0.639-0.887)	.001
LDL (mg/dL)	≤50.5	0.556	0.759	0.680 (0.539-0.822)	.021
AST (U/L)	≤430.5	0.833	0.776	0.871 (0.809-0.932)	<.001
ALT (U/L)	≤225.5	0.833	0.862	0.911 (0.855-0.966)	<.001
ALP (U/L)	≤232.0	0.667	0.828	0.805 (0.694-0.916)	<.001
Uric acid (mg/dL)	≤1.45	0.944	0.845	0.930 (0.887-0.972)	<.001
Alp/T ratio	≤8.69	0.556	0.948	0.773 (0.644-0.902)	<.001
T.bil (mg/dL)	≥23.43	0.583	0.722	0.678 (0.550-0.807)	.006
D.bil (mg/dL)	≥13.20	0.667	0.670	0.659 (0.524-0.793)	.014
GGT (U/L)	≥50.5	0.750	0.687	0.719 (0.608-0.830)	.001
AST/ALT	≥2.03	0.625	0.870	0.818 (0.725-0.912)	<.001

Abbreviations: NWALF, nonwilsonian acute liver failure; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; D.bil, direct bilirubin; FWD, fulminant Wilson's disease; GGT, gammaglutamyl transferase; Hb, Haemoglobin; PLT, platelet; T.bil, total bilirubin.

In 14 children with FWD, Coombs negative haemolytic anaemia was detected. Hb, T.bil, D.bil and indirect bilirubin (I.bil) values were compared in patients with FWD with and without haemolytic anaemia. Hb levels (7.71 g/dL, 11.79 g/dL, $P < .001$) were significantly lower and T.bil (36-16.4 mg/dL, $P = .005$), D.bil (24-11.5 mg/dL, $P = .013$) and I.bil (12-4.9 mg/dL, $P = .002$) were found to be significantly

TABLE 3 FWD scoring system according to biochemical data of the patients

	Cut-off value for FWD	Score
Age	≥4.5	1
	<4.5	0
Hb (g/dL)	≤9.15	1
	>9.15	0
PLT (10^3/mL)	≤169.5	1
	>169.5	0
Albumin (mg/dL)	≤2.95	1
	>2.95	0
Cholesterol (mg/dL)	≤97.0	1
	>97	0
LDL (mg/dL)	≤50.5	1
	>50.5	0
AST (U/L)	≤430.5	1
	>430.5	0
ALT (U/L)	≤225.5	1
	>225.5	0
ALP (U/L)	≤232	1
	>232	0
Uric acid (mg/dL)	≤1.45	1
	>1.45	0
ALP/T.bil ratio	≤8.69	1
	>8.69	0
T.bil (mg/dL)	≥23.425	1
	<23.425	0
D.bil (mg/dL)	≥13.2	1
	<13.2	0
GGT (U/L)	≤50.5	1
	>50.5	0
AST/ALT	≥2.03	1
	<2.03	0

Note: FWD diagnosis sensitivity = 0.889. Specificity = 0.879.
AUC = 0.962 (0.924-1.000) in patients with a new cut-off point ≥4.5 according to 14 laboratory variables. $P < .001$.
When we add age as the 15th variable. The best cut-off point in the total score ≥6.5 and sensitivity = 0.889 specificity = 0.914. AUC = 0.967 (0.933-1.000). $P < .001$.
According to six laboratory variables (AST, ALT, ALP, AST/ALT ratio, uric acid and Hb), a new cut-off point ≥2.5 was detected. FWD diagnosis sensitivity = 0.875. Specificity = 0.867. AUC = 0.932 (0.870-0.994). $P < .001$.
When we added age as the seventh variable, we found a new cut-off point ≥3.5. FWD diagnosis sensitivity = 0.875 specificity = 0.912.
AUC = 0.951 (0.905-0.997) $P = .024$.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FWD, fulminant Wilson's disease; GGT, gammaglutamyl transferase; Hb, haemoglobin; PLT, platelet.

higher in FWD patients with Coombs negative haemolytic anaemia than those without it. We determined the best cut-off points for the laboratory findings using ROC analysis. Accordingly, Hb ≤9.15 g/dL, PLT ≤169.5 10^3/mL, albumin ≤2.95 mg/dL, cholesterol ≤97.0 mg/dL, LDL ≤50.5 mg/dL, AST ≤430.5 U/L, ALT ≤225.5 U/L, ALP ≤232 U/L, uric acid ≤1.45 mg/dL, ALP/T.bil ≤8.69, T.bil ≥23.43 mg/dL, D.bil ≥13.2 mg/dL, GGT ≥50.5 U/L and AST/ALT ≥2.03 were determined as the best cut-off points (Table 2). Patients meeting these cut-off points were assigned one point and others were assigned zero point. We then formed a new variable consisting of the combination of 14 variables and performed a new ROC analysis. We obtained a cut-off point of ≥4.5 for FWD. The diagnostic performance of the score was characterized by a sensitivity of 0.889, a specificity of 0.879 and an AUC of 0.962 (0.924-1.000) ($P < .001$) (Tables 2 and 3).

We determined a best cut-off point of ≥4.5 for age. It had a sensitivity of 0.917, a specificity of 0.525 and an AUC of 0.665 (0.571-0.758) ($P = .011$). When we added age as the 15th variable, we obtained a best cut-off value of ≥6.5 for the total score, which had a sensitivity of 0.889, a specificity of 0.914 and an AUC of 0.967 (0.933-1.000) ($P < .001$) (Table 3).

A scoring system based only on AST, ALT, ALP, AST/ALT ratio, uric acid and Hb had a best cut-off point of ≥2.5 for FWD, which had a sensitivity of 0.875, a specificity of 0.867 and an AUC of 0.932 (0.870-0.994) ($P < .001$) (Table 3). Adding the variable of age to these six variables yielded a cut-off point of ≥3.5 with a sensitivity of 0.875, a specificity of 0.912 and an AUC of 0.932 (0.870-0.994) ($P < .001$) (Table 3).

The patients were evaluated according to the mortality scores of Nazer and Dhawan (modified Nazer prognostic score).[8,9] The Nazer score was 9.37 ± 1.48 in NWALF patients and 9.1 ± 2.08 in FWD patients. In addition, the Dhawan score was 13.01 ± 2.27 in NWALF patients and 13.5 ± 3.02 in FWD patients. There was no statistically significant difference between the two groups ($P = .426$ and .376 respectively) (Table 4).

4 | DISCUSSION

It is challenging to diagnose WD during the course of NWALF. However, due to the poor prognosis of this condition, its early diagnosis is of vital importance. It is usually difficult to obtain any useful information from the diagnostic criteria of the WD, including KF ring (examination of critical patients with a slit lamp is inconvenient), 24-hour urinary copper (collection and analysis is too time-consuming), neurological findings (neurological signs may be masked in critically ill), hepatic copper accumulation (biopsy is not feasible due to coagulation disorders in many patients, and obtaining a test result usually takes a long time), in a critical time window. Ceruloplasmin drops below 20 mg / dL in the majority of patients with WD as a result of impaired biosynthesis. The ceruloplasmin level may also decrease in NWALF.[10-12] Furthermore, 20% of children and adults with WD who carry biallelic missense mutations of the ATPB7 gene, might have normal serum ceruloplasmin levels.[13,14] In a study comprising 57 adult and paediatric WD patients, the best cut-off for ceruloplasmin to diagnose WD was 14 mg/dL with a sensitivity of 93% and a specificity of 100%.[11] Among 40

TABLE 4 Comparison of mortality scores of children with FWD and other NWALF

	NWALF	FWD	*P*
Nazer score	9.37 ± 1.48	9.1 ± 2.08	.426
Dhawan score	13.01 ± 2.27	13.5 ± 3.02	.376

Note: Independent samples test.
Abbreviations: NWALF, nonwilsonian acute liver failure; FWD, fulminant Wilson's disease.

asymptomatic WD patients, the best cut-off value of ceruloplasmin was 20 mg/dL, which had a sensitivity of 95% and a specificity of 84.5%.[12] In our study all patients had NWALF and almost identical to that reported in the literature; we found the best cut-off point as ≤19.5 for ceruloplasmin in FWD, which had a sensitivity of 0.814 and a specificity of 100.

Nazer et al[8] evaluated 27 Wilson patients and developed a mortality score based on AST, bilirubin and INR values. Accordingly, Wilson patients who have a score of ≥7 indicate a high mortality risk. The sensitivity and specificity of the Nazer scoring system was 87% and 90%, with a positive predictive value of 72%.

Dhawan et al[9] developed a New Wilson Index for Predicting Mortality score in a retrospective study of 57 symptomatic (17 FWD) paediatric WD and showed that transplantation-free mortality was higher in Wilson patients who scored ≥11.[9] This mortality index had 93% sensitivity, 98% specificity and positive predictive value of 93%. In both FWD and NWALF our patients had a Nazer score of ≥7 and a Dhawan-developed New Wilson Index for Predicting Mortality score ≥11. These results showed high mortality in both FWD and NWALF patients. There was no significant difference between the two groups for the Nazer and Dhawan scores (*P* = .491 and .101 respectively). Nazer and Dhawan's studies[8,9] are prognostic scoring. Our study can be used as a diagnostic test that can differentiate FWD from other NWALF patients based on biochemical parameters.

Urinary copper excretion is usually within normal limits among patients with asymptomatic WD or WD with mild liver disease. The reported optimal diagnostic cut-off point for basal urinary copper is 40 μg/24 h (0.65 mol)/24 h, which has a sensitivity of 78.9% and a specificity of 87.9%.[10,12] It was reported that D-penicillamine and 24-hour urinary copper excretion had a sensitivity of 12% and a specificity of 46% for asymptomatic WD.[12] We determined the best cut-off point of ≥239.5 μg/24 h (*P* < .001), which had a sensitivity of 0.917 and a specificity of 0.100 for FWD.

Forty per cent of NWALF patients suffer from renal dysfunction with reduced urine output, which limits the potential benefit of Cu measurement. Furthermore, disorders characterized by massive hepatocellular necrosis are known to increase urinary copper excretion. It was reported that there might be a wide overlap between FWD and NWALF in terms of urinary copper content.[2,15]

There is a limited number of studies in the literature that can meet the need for new diagnostic markers/methods to differentiate FWD from NWALF. El Balkhi et al[16] evaluated the levels of exchangeable Cu (CuEXC) and relative exchangeable copper (REC) (i.e. CuEXC/total copper ratio) as biomarkers for WD. Ceruloplasmin unbound copper (NCC), CuEXC and REC had 75%, 88% and 100% sensitivity. The CuEXC level is thought to correspond to the variable fraction of complex copper for albumin.[16-19] However, CuEXC can be easily modified in the presence of high copper affinity chelators such as EDTA and can be determined after incubation of serum with EDTA for 1 hour followed by ultrafiltration of diluted serum.[16] In our study, total Cu and NCC levels were measured only in WD and there was no data on serum copper levels in other NWALF patients. Since we could not compare between the two groups, we did not include them in the scoring system. In another published study,[20] the most appropriate cut-off point for total Cu and NCC levels for FWD development in Wilson patients was ≥46 and ≥67.72 μg/dL respectively. Their sensitivity was 100% and 87.5% respectively.[20] This supports the work of El Balkhi et al.[16]

There is a limited number of studies in the literature that might meet the need for novel diagnostic markers/methods to differentiate FWD from NWALF. Some researchers have reported the use of rapid and easily accessible biochemical markers as differential diagnostic markers.[2,21] Korman et al[2] studied 124 NWALF patients and 16 FWD patients (three paediatric cases) and reported that a Hb count of less than 10 g/dL had a sensitivity of 94%, a specificity of 74%; an AST/ALT ratio of greater than 2.2 had a sensitivity of 94% and a specificity of 86; and an ALP/T.bil ratio of less than 96% had a sensitivity of 94% and a specificity of 96%. Similarly, Berman et al,[21] in a series of 6 FWD patients including a paediatric patient, reported that using a cut-off point of 2.0 for ALP/T.bil could easily differentiate FWD and NWALF from all other aetiologies; they also reported that, when a cut-off point of 4.0 was used for AST/ALT ratio, a sensitivity of 83% and a specificity of 100% could be achieved. Moreover, Kolman et al[2] reported a specificity and a sensitivity both 100% with combined use of AST/ALT and ALP/T.bil.

In contrast, Eisenbach et al,[22] in their study on 7 FWD and 8 other NWALF case series, showed that AST and ALT values were significantly higher in the FWD group (*P* = .037 and *P* < .001 respectively). ALP, AST/ALT and ALP/T.bil levels were not significantly different between the two groups (*P* = .749, .266 and .363 respectively). Although there was no significant difference between the groups, the mean AST/ALT level was 2.3 ± 1.5 in FWD patients, which supported the results of Kolman et al.[2]

In accordance with our study, in the study of Eisenbach et al,[22] FWD patients had significantly younger age and lower Hb levels compared to NWALF patients. (*P* = .016 and *P* < .0001 respectively). Higher ALP and ALP/T.bil values that were revealed in the same study could be explained by the low case number and the lower mean age of FWD patients.

Our study with the largest paediatric series so far demonstrated that a cut-off point of ≤9.15 for Hb confirmed the diagnosis of FWD with a sensitivity of 66.7% and a specificity of 76.7% (*P* < .001); a cut-off point of ≥2.0316 for AST/ALT confirmed the diagnosis of FWD with a sensitivity of 62.5% and a specificity of

87% ($P < .001$); and a cut-off point of ≤8.6904 for ALP/T.bil ratio confirmed the diagnosis of FWD with a sensitivity of 55.6% and a specificity of 94.8% ($P < .001$). We attribute our higher ALP/T.bil ratio than those reported before to the fact that only paediatric patients were included in our study. Zierk et al[23] demonstrated that ALP was inversely correlated with age in 361 405 samples from 124 440 patients. However, the Hepatology Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition[10] reported that serum transaminase levels were lower and ALP (IU/L)/T.bil (mg/dL) ratio was <1 in paediatric FWD and cited the studies of Tissieres et al[24] and Sallie et al[25] to support their findings. However, when we converted bilirubin unit to µmol/L, we obtained an ALP/T.bil ratio of 0.3838 IU/µmol, which was similar to that reported previously. This supports the reported [10] ALP / T.bil ratio in patients with pediatric FWD. Chang et al [26]studied 4232 children aged 1-14 years and reported that ALT remained unchanged but AST decreased as patients got older. In our study, despite higher mean age and lower ALT and AST levels of children with FWD compared to others, AST/ALT ratio was significantly higher (1.36 ± 0.78 vs 4.26 ± 3.69; $P < .001$). We noticed that some parameters such as AST/ALT, ALP/T.bil and Hb had lower sensitivity and specificity when used alone compared to that reported previously in the literature.[2,21]

In hepatocytes, ALT is localized only in cellular cytoplasm, while AST is both cytosolic (20% of total activity) and mitochondrial (80% of total activity).[27]

The third zone of hepatic acinus has a higher concentration of AST, and whether ischaemic or toxic, damage to this site may cause greater changes in AST levels.[28] AST and ALT are enzymes that catalyse the transfer of α-amino groups from aspartate and alanine to α-keto-ketoglutaric acid groups to produce oxaloacetic and pyruvic acids which significantly contribute to the citric acid cycle respectively. Both enzymes require pyridoxal-5′-phosphate (vitamin B6) to perform this reaction. In patients with liver disease, pyridoxal-5′-phosphate deficiency may decrease ALT serum activity and contribute to an increased AST/ALT ratio.[29] We could not show vitamin B6 deficiency in our patients. However, the lack of vitamin B6 and the presence of AST in both cytoplasm and mitochondria may be the causes of AST and ALT difference between the two groups.

Haemolytic anaemia is known to decrease Hb while increasing the level of T.bil and I.bil.[30,31] To the best of our knowledge, there is no comprehensive study on Coombs negative haemolytic anaemia in FWD. In a large cohort study with Wilson's patients, Ferenci et al detected Coombs negative haemolytic anaemia in 42 of the 53 patients with FWD. However, there are no data about Hb and bilirubin values in the study.[32] But there are case-based reports in Wilson patients with and without fulminant liver failure.[33,34] Most of the studies include haemolytic anaemia due to other aetiologies such as autoimmune or drug-induced haemolytic anaemia.[35,36] In our study, Coombs negative haemolytic anaemia was present in 14 (58.3%) of the 24 FWD patients. In these patients, we found that, besides low Hb and higher T.bil and I.bil values, D.bil level was sufficiently high. This may be related to cholestasis due to increased copper exposure in FWD. Similar findings have been reported in an FWD patient diagnosed with acute haemolytic crisis.[34]

Zinc deficiency has been reported to cause low ALP levels.[37] In our study, zinc level was low in all 8 children with FWD, in whom serum zinc was studied, which may explain the low ALP in patients with FWD.

While we could not fully explain this situation, we believe that, given the variability of biochemical and haematological parameters by ageing and the aetiology, it may have been due to a greater number of the paediatric FWD patients and a greater aetiological diversity of the NWALF patients in our study than in previous reports. Therefore, unlike the previous reports, we analysed the sensitivity and specificity of 14 variables to make the diagnosis of FWD (Table 2). We assigned one point to variables meeting the cut-off point for FWD and zero point to those that did not meet the cut-off values. We re-determined a cut-off level for FWD by the help of this scoring system. According to this system, a new cut-off point of ≥5.5 determined from 14 variables yielded a sensitivity of 88.9% and a specificity of 87.9% for FWD ($P < .001$). When we added age as the 15th variable, we obtained a sensitivity of 88.9% and a specificity of 91.4 ($P < .001$).

We re-determined a cut-off point using six variables (AST, ALT, ALP, AST/ALT ratio, uric acid and Hb). A cut-off point of ≥2.5 had a sensitivity of 87.5% and a specificity of 86.7% ($P < .001$). The inclusion of age as the 7th variable resulted in a sensitivity of 87.5% and a specificity of 91.2% ($P < .001$).

We also compared ROC analysis and AUC for 4 different scoring systems. There was no statistical difference between the areas under the curve ($P > .05$). Therefore, since scoring with fewer variables is more practical, we recommend using 6 variable scoring.

After FWD is suggested by this scoring system, taking samples to confirm the diagnosis with conventional diagnostic methods and starting chelation therapy as soon as possible is recommended.

The most important limitation of our study is its retrospective design. Therefore, we could not compare our results with the recently published non-invasive rapid diagnostic markers including relative exchangeable copper (REC).[16]

However, being the largest series which compares FWD and NWALF in children and detailed statistical analysis of each biochemical parameter makes it valuable.

This study would help clinicians to decide whether or not to start chelation therapy where the results of ceruloplasmin and urine copper are not achieved quickly.

In conclusion, early diagnosis of FWD is of paramount importance for the early treatment of those patients. Our study demonstrated that biochemical markers offer almost as reliable, fast and accurate diagnosis of FWD as offered by ceruloplasmin and 24-hour urinary copper, which would allow a more rapid institution of bridging therapies such as chelation therapy and plasmapheresis to reduce serum copper level in a rapid way in order to alleviate liver injury and break the haemolytic cycle.

DISCLOSURE

The authors do not have any disclosures to report.

CONFLICT OF INTEREST

The authors have no conflict of interest or financial interest related to the manuscript to disclose.

ETHICAL APPROVAL

Prior to the start of the work, local institutional ethics committee approval was obtained. Ethics committee date: 2017 Ethical committee no: 22-2.

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