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Clinical studies

# The effects of iron and zinc status on prognosis in pediatric Wilson's disease

Şükrü Güngör[a,*], Mukadder Ayşe Selimoğlu[a], Fatma İlknur Varol[a], Serdal Güngör[b], Muhammed Mehdi Üremiş[c]

[a] Department of Pediatric Gastroenterology, Hepatology and Nutrition, İnönü University, Faculty of Medicine, Malatya,Turkey
[b] Department of Pediatric Neurology, İnönü University, Faculty of Medicine, Malatya,Turkey
[c] Department of Medicinal Biochemistry, İnönü University, Faculty of Medicine, Malatya, Turkey

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ABSTRACT

*Objectives:* Wilson's disease (WD) is a metabolic disorder leading to hepatic and extrahepatic copper deposition. Several studies have reported that besides copper (Cu), iron (Fe) and zinc (Zn) are also accumulated at varying levels in various tissues in WD. However, there is not an adequate number of studies investigating the effects of Fe and Zn status on WD presentation and prognosis. We aimed to evaluate serum levels of ferritin (SFr), copper (SCu), and zinc (SZn) in WD and determine their role in disease presentation and prognosis.

*Materials-Method:* We retrospectively reviewed the medical records of 97 pediatric patients with WD who were diagnosed and followed at İnönü University Pediatric Gastroenterology, Hepatology and Nutrition Department between January 2006 and May 2017. Serum Cu and Zn levels were analyzed by using flame atomic absorption spectrophotometer. Ferritin was analyzed by chemiluminescence immunoassay method.

*Results:* Analysis of serum levels of the elements according to the type of presentation, there was no significant difference between the groups for ceruloplasmin. However, SCU, FSCu, SFr and 24 h urinary copper levels were significantly higher ($p = 0.002$, $p = 0.003$, $p = 0.023$ and $p < 0.001$, respectively) and SZn and CSZn levels were significantly lower (fulminant WD). $p < 0.001$, $p < 0.001$). There was a positive correlation between SFr, SCu serum levels and mortality scores (respectively, r: 0.501, 0.564 for PELD, r:0.490, 0.504 for MELD, r: 0.345, 0.374 for Dhwan), and a negative correlation between SZn level and mortality scores. (r:-0.650 for PELD, r:-0.703 for MELD, r:-0.642 for Dhwan) We used the ROC curves to determine the worst prognosis for fulminant Wilson disease. According to these limit values, we found that the sensitivity and specificity of FWD development was significantly higher. (for SZn sensitivity of 91.5%, a specificity of 100%, $p = < 0,001$, for SCu predicted FWD development with a sensitivity of 100%, a specificity of 73.7%, $p = < 0,001$, for SFr predicted FWD development with a sensitivity of 92.9%, a specificity of 66.2%, $p < 0,001$)

*Conclusion:* Our study suggests that SFr, SCu, SZn levels might have prognostic importance for WD.

## 1. Introduction

Wilson's disease (WD) is a metabolic disorder leading to hepatic and extrahepatic copper deposition due to mutations in ATP7B encoding P-type ATPase [1–5]. Its diagnosis is usually made with combined assessment of clinical and laboratory findings and genetic tests [1–3]. The determination of copper concentration in liver biopsy is planned when the other criteria are not discriminating [6]. A hepatic copper (Cu) concentration of 250 μg/g or greater measured using flame atomic absorption spectroscopy (FAS) is considered diagnostic for WD [1]. Several studies have reported that besides copper (Cu), iron (Fe) and zinc (Zn) are also accumulated at varying levels in various tissues in WD and have suggested a pathogenetic relationship between such accumulation and WD [1,7,8]. However, there is not an adequate number of studies investigating the effects of Fe and Zn status on WD presentation and prognosis. Hence, we aimed to evaluate serum levels of ferritin (SFr), copper (SCu), and zinc (SZn) in WD and determine their role in disease presentation and prognosis.

## 2. Materials and method

We retrospectively reviewed the medical records of 97 pediatric patients with WD who were diagnosed and followed at İnönü University Pediatric Gastroenterology, Hepatology and Nutrition Department

* Corresponding author at: İnönü University, Faculty of Medicine, Dept. of Pediatrics, Malatya, Turkey.
*E-mail addresses:* sukru.gungor@yahoo.com (Ş. Güngör), ayseselimoglu@hotmail.com (M.A. Selimoğlu), drivarol@yahoo.com (F.İ. Varol), serdal.gungor@inonu.edu.tr (S. Güngör), mmuremis@hotmail.com (M.M. Üremiş).

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between January 2006 and May 2017.

The diagnosis of WD was made using the scoring system developed at the 8th International Meeting on Wilson Disease held in Leipzig in 2001 [9]. Clinical presentations were defined as follows [10–14]:

Asymptomatic WD: Asymptomatic children who were found having increased transaminase levels and/or hepatomegaly levels during routine check-ups or family screening.

Acute WD: WD with acute-onset liver dysfunction detected by clinical and laboratory investigations in a patient without a history of liver disease.

Fulminant WD: Encompasses WD with acute liver failure detected on the basis of the Pediatric Acute Liver Failure Study Group Criteria.

1) children with no evidence of chronic liver disease, 2) biochemical evidence of acute liver injury, 3) hepatic-based coagulopathy (defined as Prothrombin time (PT) ≥15 s or INR ≥ 1.5 not corrected by vitamin K in the presence of hepatic encephalopathy; or PT ≥ 20 s or INR ≥ 2.0 regardless of presence or absence of clinical encephalopathy).

Chronic liver disease presentation: Patients with previously known/unknown WD, presenting with clinical and laboratory chronic liver failure findings.

Neurological WD: Patients presenting with neurological symptoms.

All data of the patients included in the study were pre-treatment data at the time of diagnosis. Patients without pre-treatment data were not included in the study. Patients were asked for use of iron, zinc or copper chelation and the cases who did not have these treatments were included.

We could provide initial SZn, SCu, and SFr values of 56, 27, and 44 patients from the medical records. Serum Cu and Zn levels were analyzed by using flame atomic absorption spectrophotometer. The analysis of serum and urine samples were performed in the recommended conditions of the manufacturer. Samples were diluted 10 times for Cu, and Zn determination using 1% $HNO_3$. Commercial Cu and Zn calibrators were used as standards (1,000 mg/L, Perkin Elmer, USA) by serial dilutions, and samples were evaluated according to a standard curve. Each result was corrected for the appropriate reagent used and matrix blanks. For the accuracy of trace element concentrations, each sample was measured two times for each element analysis. Deionized water was used to clean the chamber and zero control for each analysis. Stock standard metal solutions for every metal were used for positive control and were tested every 5 samples to ensure reliability of measurement. 1% $HNO_3$ was used for negative control and were tested every samples. Ferritin was analyzed by chemiluminescence immunoassay method. Using ADVIA Centaur XP (Siemens Diagnostics, Deerfield, IL, USA). Ceruloplasmin was measured by immunoturbidimetric method by Integra 800 system (CERU, Roche Diagnostics, Mannheim, Germany). In order to evaluate the imprecision and accuracy of laboratory tests in clinical biochemistry laboratories, internal and external quality controls were studied at different levels. Internal control was performed with serum supplied by Bio-Rad Laboratories for Ferritin. Internal control serum provided by Roche Diagnostics was used for Ceruloplasmin. Internal control was performed with whole blood supplied by Recipe Chemicals + Instruments GmbH. When the internal control was outside the accepted range, new calibration was implemented.

Zinc deficiency was accepted if the serum zinc level was below 70 μg / dl. (Normal values is 70–125 μg / dl) ([15])

Corrected Serum Zn (CSZn) level was calculated as follows:

CSZn = 0.25 x Zn Control + Albumin Control / Albumin Patient x Zn Patient - 0.25 x Zn Control [16,17].

The serum ferritin level was defined as deficient if it was ≤ 30 ng / mL, and normal level was 31–99 ng / mL and high ferritin level was ≥ 100 ng / mL. ([18])

Free copper level > 25 μg/dl was considered as an increased level (normal value is < 15 μg/dl [12].

Free Serum Copper Level (FSCu) was calculated by the following formula:

(FSCu) = Copper – 3 x ceruloplasmin level.

Normal range is 1.3–1.9 μmol/L [12].

Hepatic disease severity was assessed according to PELD (Pediatric End-Stage Liver Disease; for children < 12 yr.), MELD (Model for End-Stage Liver Disease; for children ≥12 yr.), CHILD, and Dhwan scoring systems [18–20]

Data were statistically analyzed with Statistical Package for the Social Sciences for Windows (SPSS Inc, Chicago) 16.0. 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. When significant differences were observed between the groups based on the post hoc analyses, either the Tukey or Scheffe was used to determine the differences between the groups. A Chi-squared test was used to compare the categorical variables. We used the Pearson correlation test to measure the statistical relationship between two continuous variables. Statistical significance was defined as $p < 0.05$.

This study was approved by the local ethics committee (Date 2017, No: 22-2).

## 3. Results

SZn, SCu, and SFr levels had been measured in 57 (58.7%) patients (mean age: 9.0 ± 3.2 yr.), 27 (28.7%) patients (mean age: 9.4 ± 3.1 yr.), and 44 (46.8%) patients (mean age of 8.8 ± 2.9 yr.), respectively. An analysis of the serum levels of elements based on the presentation type showed that SCu, FSCu, and SFr levels were significantly higher ($p = 0.002$, $p = 0.003$, and $p = 0.023$, respectively) whereas SZn and CSZn levels were significantly lower ($p < 0.001$, $p < 0.001$, respectively) in fulminant WD than other presentation types. There was no significant difference between the ceruloplasmin levels with respect to presentation type ($P = 0.924$) The 24 -h urinary copper level was significantly higher in fulminant Wilson patients than in the other Wilson patients. (P = < 0,001) (Table 1).

Serum levels of Zn, Cu, and Fr were evaluated with respect to the disease severity scores and found that SFr and SCu levels were higher in patients having ≥12 points in the PELD score compared to those with a score of < 12 ($p = 0.20$, and $p < 0.001$, respectively). SZn and CSZn

**Table 1**
Evaluation of serum element levels in children with Wilson's Disease according to the presentation.

| | Asymptomatic | Fulminant | Chronic | NeuroWilson | p |
|---|---|---|---|---|---|
| SCu (μg/dl) (27) | 24.13 ± 20.31 | 126.75 ± 80.68 | 45.29 ± 33.00 | 38.25 ± 22.60 | 0.002 |
| FSCu (μmol/L) (27) | 23.90 ± 20.18 | 126.39 ± 80.70 | 45.11 ± 33.03 | 38.25 ± 27.49 | 0.003 |
| SZn (μg/dl) (56) | 89.10 ± 27.10 | 27.8 ± 11.73 | 56.13 ± 10.96 | 68.33 ± 21.44 | < 0.001 |
| DSZn (μmol/L) (56) | 81.42 ± 27.76 | 38.95 ± 12.41 | 79.73 ± 27.56 | 69.50 ± 26.90 | < 0.001 |
| SFr(ng/mL) (44) | 57.43 ± 35.96 | 1503.50 ± 2280.44 | 262.75 ± 264.13 | 67.10 ± 43.57 | 0.023 |
| Cp (g/L) (97) | 0.106 ± 0.076 | 0.110 ± 0.048 | 0.102 ± 0.090 | 0.12 ± 0.15 | 0.924 |
| 24 -h urine copper | 380.7 ± 403.2 | 1605.34 ± 1660.94 | 799.96 ± 1000.89 | 562.36 ± 334.78 | < 0.001 |

SCu: Serum copper level, FSCu: Free Serum Copper Level, Cp: Ceruloplasmin, SZn: Serum zinc level, DSZn: Adjusted serum zinc level, SFr: Serum ferritin level.

**Table 2**
Evaluation of serum element levels according to prognostic scores.

| PELD | | | | |
|---|---|---|---|---|
| | **< 12** | | **≥12** | **p** |
| SFr (ng/mL) (44) | 58.43 ± 43.26 | | 833.79 ± 1696.25 | 0.020 |
| SZn (µg/dl) (56) | 89.15 ± 27.55 | | 47.55 ± 18.30 | < 0.001 |
| DSZn (µmol/L) (56) | 83.76 ± 26.85 | | 61.34 ± 28.30 | 0.004 |
| SCu (µg/dl) (26) | 26.75 ± 26.61 | | 80.50 ± 69.62 | 0.009 |
| FSCu (µmol/L) (26) | 26.495 ± 26.48 | | 82.75 ± 70.86 | 0.009 |
| Cp (**g/L**) (81) | 0.968 ± 0.07 | | 0.12 ± 0.09 | 0.247 |
| **MELD** | | | | |
| | **< 19** | | **≥19** | **P** |
| SFr (ng/mL) (40) | 57.93 ± 40.12 | | 994.22 ± 1829.45 | 0.020 |
| SZn (µg/dl) (50) | 82.77 ± 26.95 | | 40.57 ± 16.31 | < 0.001 |
| DSZn (µmol/L) (50) | 78.14 ± 27.41 | | 61.10 ± 31.69 | 0.048 |
| SCu (µg/dl) (23) | 35.58 ± 30.43 | | 98.83 ± 77.24 | 0.019 |
| FSCu (µmol/L) (23) | 35.16 ± 31.88 | | 98.54 ± 77.19 | 0.020 |
| Cp (**g/L**) (82) | 0.11 ± 0.10 | | 0.11 ± 0.07 | 0.880 |
| **CHILD** | | | | |
| | **Class A** | **Class B** | **Class C** | **P** |
| SFr (ng/mL) (43) | 58.06 ± 39.61 | 146 ± 155.43 | 1024.91 ± 1864.03 | 0.091 |
| SZn (µg/dl) (55) | 85.1 ± 26.52 | 57.67 ± 11.13 | 40.37 ± 17.76 | < 0.001 |
| DSZn (µmol/L) (55) | 75.78 ± 26.41 | 79.62 ± 28.07 | 61.12 ± 32.11 | 0.176 |
| SCu (µg/dl) (26) | 41.63 ± 35.32 | 21.60 ± 12.74 | 94.00 ± 75.98 | 0.043 |
| FSCu (µmol/L) (26) | 41.18 ± 35.31 | 21.37 ± 12.75 | 98.54 ± 77.19 | 0.032 |
| Cp (**g/L**) (89) | 0.12 ± 0.10 | 0.08 ± 0.05 | 0.11 ± 0.07 | 0.424 |
| **Dhwan** | | | | |
| | **< 10** | | **≥10** | **P** |
| SFr (ng/mL) (43) | 58.04 ± 38.04 | | 1036.40 ± 1858.60 | 0.020 |
| SZn (µg/dl) (55) | 80.54 ± 27.73 | | 43.80 ± 15.94 | < 0.001 |
| DSZn (µmol/L) (55) | 74.34 ± 26.59 | | 68.99 ± 31.27 | 0.506 |
| SCu (µg/dl) (26) | 38.17 ± 28.87 | | 86.07 ± 78.13 | 0.048 |
| FSCu (µmol/L) (26) | 37.98 ± 30.24 | | 85.78 ± 78.07 | 0.051 |
| Cp (**g/L**) (90) | 0.11 ± 0.94 | | 0.11 ± 0.07 | 1.000 |

SCu: Serum copper level, FSCu: Free Serum Copper Level, Cp: Ceruloplasmin, SZn: Serum zinc level, DSZn: Adjusted serum zinc level, SFr: Serum ferritin level

levels, on the other hand, were significantly lower in patients with a score of ≥12 compared to those with a score < 12 ($p < 0.001$, $p = 0.004$, respectively). There was no significant difference between two groups with respect to ceruloplasmin levels ($p = 0.247$) (Table 2).

SFr, SCu, and FSCu levels were significantly higher in patients with a MELD score of ≥19 compared to those with a MELD score < 19 ($p = 0.020$, $p = 0.019$, $p = 0.020$, respectively). We found a significantly lower SZn level and corrected SZn level in patients with a MELD score of ≥19 than those with a MELD score of < 19 ($p < 0.001$ and $p = 0.048$, respectively). Ceruloplasmin level was not significantly different between two groups ($p = 0.880$) (Table 2).

A comparison of the elements's serum levels by the Child-pugh (CHILD) score showed that SCu and FSCu levels were significantly higher in CHILD class C group compared to the other groups ($P = 0.043$, $P = 0.032$). SZn level, on the other hand, was significantly lower in the CHILD class C group than the other groups ($P < 0.001$). Ceruloplasmin, SFr, and CSZn levels were not significantly different among study groups ($p = 0.424$, $p = 0.091$, and $p = 0.176$, respectively) (Table 2).

A comparison of the serum levels of the studied parameters according to Dhwan Prognostic Index Score showed significantly higher SFr and Scu levels in patients with a score of ≥10 compared to those with a Dhwan score of < 10 ($p = 0.019$ and $p = 0.048$, respectively). FSCu level was also higher though not statisticalally different ($p = 0.051$). We found a significantly lower SZn level in patients with a Dhwan score of ≥10 than those with a Dhwan score of < 10 ($p < 0.001$). Corrected SZn and ceruloplasmin levels were similar in both groups ($p = 0.153$ and $p = 1.000$) (Table 2).

Pearson correlation analysis revealed that there was a significant negative correlation between SZn level and PELD, MELD, and Dhwan scores (r: −0.650, $p < 0.001$; r: −0.703, $p < 0.001$; and r: −0.642, $p < 0.001$, respectively). While there was also a significantly negative correlation between corrected SZn level and PELD, MELD scores (r: −0.362, $p = 0.014$; and r: −0.379, $p = 0.007$, respectively), no significant correlation was found between CSZn and Dhwan score (r. −0.197, $p = 0.153$). There was a significant positive correlation between serum ferritin level and PELD, MELD, and Dhwan scores (r: 0.501, $p = 0.001$; r: 0.490; $p = 0.001$; r: 0.345, $p = 0.024$, respectively). We found a significant positive correlation between serum Cu and FSCu levels and PELD, and MELD scores (r: 0.564, $p = 0.005$; r: 0.504, $p = 0.012$, respectively; and r: 0.560 $p = 0.007$; r: 0.502, $p = 0.015$, respectively). There was a statistically non-significant, weak, positive correlation between Dhwan score and SCu level (r: 0.374, $p = 0.056$). Dhwan score and FSCu level showed a non-significant, weak, positive correlation, as well (r: 0.371, $p = 0.068$) (Table 3).

We determined the best cut-off points using ROC curves to predict the development of fulminant Wilson's disease, which portends the worst prognosis. The best cut-off point of ≤47 mg/dl for SZn predicted FWD development with a sensitivity of 91.5%, a specificity of 100%,

**Table 3**
Correlation between serum element levels and prognostic scores.

| | | PELD | MELD | Dhwan |
|---|---|---|---|---|
| SZn (µg/dl) | r | −0.650 | −0.703 | −0.642 |
| | P | < 0.001 | < 0.001 | < 0.001 |
| DSZn (µmol/L) | r | −0.362 | −0.379 | −0.197 |
| | P | 0.014 | 0.007 | 0.153 |
| SFr (ng/mL) | r | 0.501 | 0.490 | 0.345 |
| | P | 0.001 | 0.001 | 0.024 |
| SCu (µg/dl) | r | 0.564 | 0.504 | 0.374 |
| | P | 0.005 | 0.012 | 0.056 |
| FSCu (µmol/L) | r | 0.560 | 0.502 | 0.371 |
| | P | 0.007 | 0.015 | 0.068 |

Pearson Correlation test.
SCu: Serum copper level, Cp: Ceruloplasmin, SZn: Serum zinc level, DSZn: Adjusted serum zinc level, SFr: Serum ferritin level.

**Table 4**
Determination of cut-off levels of serum element levels for Fulminan Wilson's disease.

| Variable | Cut-off value for Fulminan Wilson disease | Sensitivity | Specificity | AUC (95% C.I.) | p value for AUC |
|---|---|---|---|---|---|
| SZn (μg/dl) (56) | ≤47 mg/dl | 0.915 | 1.000 | 0.955-1.000 | < 0.001 |
| DSZn (μmol/L) (56) | ≤57.92 mg/dl | 0.822 | 1.000 | 0.885-1.000 | < 0.001 |
| SCu (μg/dl) (27) | ≥46 μg/dl | 1.00 | 0.737 | 0.837-1 | 0.001 |
| FSCu (μmol/L) (27) | ≥67.72 μg/dl | 0.875 | 1.000 | 0.833-1.000 | 0.001 |
| SFr (ng/mL) (44) | ≥93.5 ng/ml | 0.929 | 0.662 | 0.773-0.989 | < 0.001 |

SCu: Serum copper level, FSCu: Free serum copper level, Cp: Ceruloplasmin, SZn: Serum zinc level, DSZn: Adjusted serum zinc level, SFr: Serum ferritin level.

and an AUC of (95% C.I.) (0.955–1.000) ($p < 0.001$). The best cut-off point of ≤57.92 mg/dl for CSZn predicted FWD development with a sensitivity of 82.2%, a specificity of 100%, and an AUC of (95% C.I.) (0.885–1.000) ($p < 0.001$). The best cut-off point of ≥46 mg/dl for SCu predicted FWD development with a sensitivity of 100%, a specificity of 73.7%, and an AUC of (95% C.I.) (0.837–1) ($p = 0.001$). The best cut-off point of ≥67.715 μg r/dl for FSCu predicted FWD development with a sensitivity of 87.5%, a specificity of 89.9%, and an AUC of (95% C.I.) (0.833–1) ($p = 0.001$). The best cut-off point of ≥ 93.5 ng/ml for SFr predicted FWD development with a sensitivity of 92.9%, a specificity of 66.2%, and an AUC of (95% C.I.) (0.837–1) ($p < 0.001$) (Table 4).

The best cut-off point of ≥69, 5 mg / dl for SZn predicted asymptomatic WD development with a sensitivity of 64.2%, a specificity of 84.4%, and an AUC of (95% C.I.) (0.805–0.969) ($p < 0.001$). This analyse could not predict asymptomatic WD development for CSZn. ($p = 0.069$) The best cut-off point of ≤28 mg / dl for SCu predicted asymptomatic WD development with a sensitivity of 84.2%, a specificity of 75%, and an AUC of (95% C.I.) (0.644-0.994) ($p = 0.010$). The best cut-off point of ≤31.89 μg r / dl for FSCu predicted asymptomatic WD development with a sensitivity of 77.8%, a specificity of 75%, and an AUC of (95% C.I.) (0.636-0.989) ($p = 0.012$). The best cut-off point of ≤112 ng / ml for SFr predicted asymptomatic WD development with a sensitivity of 53.1%, a specificity of 91.7%, and an AUC of (95% C.I.) (0.626-0.905) ($p = 0.007$).

The best cut-off point of ≤69, 5 mg / dl for SZn predicted chronic WD development with a sensitivity of 50%, a specificity of 76.7%, and an AUC of (95% C.I.) (0.588–0.850) ($p = 0.007$). It could not predict chronic WD development for CSZn, SCu, FSCu and SFr. ($p = 0.720$, $p = 0.678$, $p = 0.623$, $p = 0.797$, respectively).

We wanted to determine the best breakpoints using the ROC curves to predict Neurologic WD development. However, ROC analysis could not predict the development of Neurologic WD for SZn, CSZn, SCu, FSCu and SFr. ($p = 0.716$, $p = 0.808$, $p = 0.517$, $p = 0.495$, $p = 0.120$ respectively).

## 4. Discussion

It was shown that serum concentrations of some trace elements including zinc decrease as a result of tissue redistribution in critically ill patients [22–24]. It has been reported that zinc deficiency increases inflammation and zinc treatment contributes to the anti-inflammatory process. [25–27]. Other studies emphasized an increased prevalence of Zn deficiency in chronic liver disease; furthermore, Zn has also been implicated in the pathogenesis of cirrhosis during the course of chronic liver disorders [22,27–32]. In a study on 18 children with WD, who had hepatic and/or neurological involvement, the patient group had a significantly lower Zn level compared to the healthy controls [33]. Iorio et al. [34] proposed a possible link between Zn levels and severity of WD. In agreement with the previous studies, our study demonstrated a significantly lower SZn level in FWD compared to the other types of presentations. When we calculated corrected SZn (CSZn) level to eliminate the effect of reduced albumin level on SZn concentration in inflammation, we still found a significantly lower SZn level in FWD compared to other presentations. This suggests that, in addition to its role in WD treatment, possible role of Zn in the pathogenesis of FWD should be elucidated. Impaired copper metabolism causes oxidative stress in WD [35,36]. On the other hand, hepatic oxidative stress has been shown to impair Zn transporters either directly or by altering their levels of transcription, as shown in alcoholic liver disease [37]. Zn treatment was shown to improve oxidative parameters in WD [38]. Zn supplementation was shown to increase metallothionine induction and thus reduce copper absorption [39]. It is theoretically possible that copper causes a drop in SZn levels in WD by preventing its absorption. There is a paucity of studies exploring the pathogenetic mechanisms in respect with the severity of WD. According to the study conducted on the largest series as ever, Zn level might be a marker of disease severity in WD [22]. Consistent with this hypothesis, our study showed a negative correlation between SZn, CSZn levels and prognostic scores. We noticed an inverse correlation between SZn levels and the scores of MELD, PELD, CHILD, and Dhwan. We determined the best cut-off points of as ≤47 mg/dl (sensitivity 91.5% and specificity 100%) and ≤57.917 mg/dl (sensitivity 82.2% and specificity 100%) for SZn and CSZn levels, respectively, for the development of FWD. This shows, in line with the previous reports, that SZn level might be an important prognostic marker for WD. There is, however, an ongoing need for large-scale prospective studies on this subject. There is a paucity of studies investigating the prognostic value of serum cupper level in WD. Zhou et al [40] reported that, although liver functions affected serum copper level in hepatic type WD, they were not related to symptom severity in neurological WD. Furthermore, the authors emphasized that a high SCu level was a marker of a worse prognosis, and SCu level may be an useful marker for therapeutic monitoring. Our study also demonstrated that SCu and free SCu levels were significantly higher in FWD ($p < 0.001$), and there is a positive correlation between prognostic scores and serum Cu values. Although it is well known that copper and zinc concentrations generally move in the opposite direction, these results support the hypothesis that the higher SCu level and low SZn level may be a prognostic marker in Wilson patients.

To our best knowledge, no study has ever investigated the prognostic value of serum ferritin level in WD. However, we may extrapolate the findings of studies performed on other liver disorders. Kowdley et al [41] demonstrated that increased serum ferritin level worsened histological activity of nonalcoholic steatohepatitis (NASH) and was an independent determinant of advanced hepatic fibrosis among patients with nonalcoholic fatty liver disease (NAFLD). Many similar studies have stressed that SFr level was an independent predictor of liver injury and severe liver fibrosis among patients with NAFLD [42–45]. Another study [46] reported that SFr level might be a determinant of fibrosis with moderate sensitivity and specificity (65% sensitivity and 60% specificity) among patients with NAFLD. Our study showed that serum ferritin level was significantly higher in FWD than the other presentations ($p = 0.023$) and there was a positive correlation between prognostic scores and SFr level. ROC analysis revealed that the best cut-off value of SFr to predict FWD was ≥93.5 ng/ml, which had a sensitivity of 92.9% and a specificity of 66.2% ($p < 0.001$). This suggests that SFr level might be an important marker to predict progression of Wilson disease to FWD.

Since our study was retrospective, some factors such as history of drug use (with the exception of iron, zinc and copper chelators), diet

and infection for all element levels were not questioned. In addition, our study did not include a control group formed by the data of unaffected individuals because it was a retrospective study. However, the subjective nature of the data was reinforced by the reliance on the assessment of disease severity-PELD, MELD etc. It deserves attention due to being one of the rare studies stressing the importance of these three elements as prognostic markers. It may also shed light on new randomized controlled trials and efforts to develop prognostic scores.

In conclusion, our study suggests that SFr, SCu, SZn levels might have prognostic importance for WD. Therefore, close monitoring of their levels, together with other prognostic factors, would make an important contribution to the efforts for determining new management strategies.

## Conflicts of interest

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

## Financial disclosure

The authors have no financial relationships relevant to this article 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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