ORIGINAL PAPER

DOI: http://dx.doi.org/10.15403/jgld-5671

Iron and Copper Liver Concentrations in Wilson Disease

Patrick L. Day[1], Ria Fyffe-Freil[2], Patrick Vanderboom[1], Grant Spears[3], Kirk J. Wangensteen[4], Joshua Bornhorst[1], Sara Hassan[5], Paul J. Jannetto[1]

1) Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN;
2) Department of Pathology, Microbiology and Immunology, University of Nebraska Medical Center, Omaha, NE;
3) Department of Quantitative Health Sciences, Mayo Clinic, Rochester, MN;
4) Division of Gastroenterology and Hepatology, Department of Medicine, Mayo Clinic, Rochester, MN;
5) Division of Pediatric Gastroenterology, Hepatology and Nutrition, Mayo Clinic, Rochester, MN, USA

Address for correspondence:
Patrick L. Day
Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA
day.patrick@mayo.edu

Received: 25.04.2024
Accepted: 23.09.2024

ABSTRACT

Background & Aims: Wilson disease (WD) results in the defective incorporation of copper into ceruloplasmin as well as decreased biliary copper excretion. Secondary iron overload has also been associated with WD; however, the prevalence is currently unknown. This study aims to determine the prevalence of potential secondary iron overload in patients suspected to have WD. The secondary aim was to determine whether common laboratory tests were associated with liver copper concentrations or the need for liver transplantation in a subset of patients with confirmed WD.

Methods: Using our institution's laboratory information system, 197 patients with liver copper concentrations > 250 mcg/g were identified who also had a concurrent liver iron concentration available. Correlations between copper, iron, and hepatic iron index were performed by log-transforming the data and then using the Pearson method. Furthermore, in a subpopulation of ten patients clinically confirmed to have WD, various laboratory test values were evaluated to determine associations with liver copper concentration or liver transplantation.

Results: There was no significant association between copper and iron liver tissue concentrations (p=0.84). However, 13 (8%) patients aged 13 or older had a hepatic iron index >1.0 which may indicate secondary iron overload. Furthermore, in clinically confirmed WD patients, hemoglobin and hematocrit were inversely associated with liver copper concentrations (p=0.036).

Conclusions: Iron overload can be detected in liver tissues with elevated copper concentrations characteristic of WD Furthermore, in WD, low hemoglobin and hematocrit values were associated with elevated liver copper concentration. Clinicians should consider the possibility of secondary iron overload and/or anemia in patients with WD.

Key words: Wilson disease – copper – iron – secondary iron overload.

Abbreviations: AFP: alpha-fetoprotein; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Hb: hemoglobin; HII: hepatic iron index; RBC: red blood cell; WD: Wilson disease.

INTRODUCTION

Wilson disease (WD) is an autosomal recessive hereditary disorder that results from pathogenic variants in *ATP7B* leading to impaired copper elimination [1]. Deficiency of ATP7B protein results in both the defective incorporation of copper into ceruloplasmin and decreased copper excretion into the bile, ultimately leading to copper accumulation in the liver and other organs [1, 2]. Clinical presentations include hepatic and neuropsychiatric manifestations [1-3]. In severe cases, it may present with decompensated hepatic failure necessitating urgent liver transplantation [4]. Diagnostic evaluation includes measurement of ceruloplasmin, 24-hour urine copper collection, hepatic copper quantification on liver biopsy, slit lamp examination for assessment of Kayser Fleischer rings or sunflower cataracts, and genetic testing for variants in *ATP7B* [2]. Low serum ceruloplasmin levels (<200 mg/L) are present in 95% of affected patients [5]. 24-hour urine copper excretion increases, serum copper is low, and hepatic aminotransferases (e.g. aspartate aminotransferase and alanine aminotransferase) are traditionally elevated in WD [2]. In addition, liver tissue copper is often elevated with copper values of 250-1000 mcg/g dry weight being suggestive of possible WD and a value > 1000

mcg/g dry weight being strongly suggestive of WD [6]. Clinical scores and diagnostic algorithms are available to navigate the investigative journey [1, 2].

The clinical consequences of reduced ceruloplasmin in WD are two-fold. First, ceruloplasmin is thought to bind around 95% of copper found in blood plasma making it essential for copper transport throughout the body [7]. With reduced ceruloplasmin, copper excretion and transport are limited. This can lead to copper accumulation in the liver and result in progressive hepatic injury. Second, copper loaded ceruloplasmin (holoceruloplasmin) is necessary for proper ferroxidation. Ferroxidation is the process by which an electron is removed from ferrous iron (Fe^{2+}) to make ferric iron (Fe^{3+}) [8]. In humans, only ferric iron is loaded onto transferrin, the major iron transport protein [8]. Thus, if there are low levels of holoceruloplasmin, as is commonly seen in WD, ferroxidation and iron transportation are disrupted. This can result in decreased circulating iron as well as increased iron stores and accumulation of iron in organs [8]. Accumulation of iron in organs is known as iron overload or hemochromatosis. Hereditary hemochromatosis is caused by variants in the gene *HFE* that result in accumulation of iron in organs such as the liver and heart [9, 10]. However, there are numerous conditions, including WD, that are thought to cause secondary iron overload which can result in similar symptoms to hereditary hemochromatosis [8, 9]. Currently, the prevalence of secondary iron overload in patients with WD is unknown and monitoring of iron is often not considered a part of routine screening in patients with WD [8].

Our clinical laboratory routinely measures copper and iron in liver tissue, providing this service for our institution and other hospitals worldwide. The availability of laboratory results enabled us to investigate the associations between liver copper concentrations and possible secondary iron overload in suspected and confirmed patients with WD. By analyzing liver biopsy copper and iron concentrations, our primary goal was to investigate whether associations exist between liver copper concentrations and liver iron concentrations in patients with suspected WD. Secondarily, by analyzing a subset of tissue samples from patients with complete medical records and a clinical diagnosis of WD, we aimed to determine whether copper and iron liver tissue levels are associated with liver transplantation as an outcome. Finally, in patients confirmed to have WD, we aimed to investigate possible relationships between commonly ordered laboratory test values, liver copper concentrations and liver transplantation, to determine whether any common laboratory values help predict liver copper concentrations or the need for liver transplantation.

METHODS

Patient Sample Selection and Data Retrieval

This study was approved by our institution's Institutional Review Board as a minimal risk study (IRB # 21-011601).

We identified 197 unique liver biopsy samples analyzed between June 2012 and January 2023 that had a copper liver tissue value of > 250 mcg/g dry tissue, which is suggestive of WD, by querying our laboratory information system. Additionally, all 197 liver biopsy samples had iron liver tissue concentration values and an associated age and gender. Since age and iron concentration were available for each patient, the hepatic iron index (mcmol/g/yr of age) could be calculated by dividing the iron liver biopsy result (mcg/g) by 56 (molecular weight of iron) and dividing this result by the age of the patient. The hepatic iron index would be calculated for liver biopsy samples acquired from patients 13 years of age or older as it is not recommended to calculate the hepatic iron index on biopsy samples acquired from patients less than 13 years of age.

Of these 197 liver biopsy samples, ten liver biopsy tissue samples were from patients seen at our institution who were confirmed to have WD by clinical criteria according to their medical record. The electronic medical record was used to confirm the diagnosis of WD, evaluate the laboratory tests performed during the diagnosis and treatment of WD and determine whether and when they underwent liver transplantation.

Laboratory Test Selection

The following clinical laboratory test values were extracted from the electronic medical record of the confirmed WD patients (n=10). These laboratory tests were selected because they may be ordered in the investigation or monitoring of suspected WD or other liver disorders. All laboratory test values preceded the liver transplant if there was indeed a transplant. Additionally, all laboratory values used for this study were acquired within 30 days of the liver biopsy. The laboratory tests used in these analyses and the established reference ranges are detailed in the Supplementary file and are further discussed below.

A copper liver tissue concentration of 250-1000 mcg/g dry weight is suggestive of possible WD. Furthermore, a copper liver tissue concentration of > 1000 mcg/g dry weight is strongly suggestive of WD [6]. Hepatic iron concentrations 3000-10,000 mcg/g dry weight are seen in mild to moderate iron overload [11]. As iron accumulates in the liver normally with aging, the hepatic iron index (HII) was also calculated for each liver tissue sample. The normal range is less than 1.0 and patients heterozygous for *HFE* variants often have an HII ranging from 1.0 to 1.9 [12].

Ferritin concentrations are directly proportional to total iron stores in the body, thus are useful in the evaluation of iron status. Patients with iron deficiency anemia usually have serum ferritin concentrations lower than healthy subjects while patients with iron overload have serum ferritin concentrations much higher than normal [13, 14].

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma are useful for the diagnosis and monitoring of liver disease associated with hepatocyte necrosis [15]. Alpha-fetoprotein (AFP) is a tumor marker that aids in the diagnosis of hepatocellular carcinoma [16]. Alkaline phosphatase (ALP) in plasma can be used in the diagnosis of hepatobiliary disease and ALP is often greatly increased in extrahepatic obstruction [15]. Bilirubin is a frequently used test to assess liver function and is used to evaluate a wide range of diseases affecting various stages of bilirubin metabolism [15].

The red blood cell (RBC) count, hemoglobin (Hb) and hematocrit are commonly ordered laboratory tests that are

used as a screening tool to confirm a hematologic disorder and to establish or rule out a diagnosis [15].

Statistical Analysis

Summary statistics of continuous test results were reported as medians, with either range or interquartile range as specified. Comparisons of continuous data were made using the nonparametric Wilcoxon rank-sum test. Correlations between copper, iron, and HII were performed by log-transforming the data to adjust for right-skewness, and then using the Pearson method. All analysis was performed using R statistical software, version 4.2.2.

RESULTS

Liver Biopsy Sample Population

In this population of 197 liver biopsy samples, the median age of the patient at time of biopsy was 24 years of age (range 0-68). Ninety-one (46.2%) biopsy samples were from females and 106 (53.8%) were from males. As is shown in Fig 1, when comparing the copper liver tissue mcg/g to the iron liver tissue -mcg/g, there was no significant correlation (Pearson correlation coefficient = -0.015; p=0.84) between copper and iron liver tissue concentrations in the study population. Because it is not common to report HII on children < 13 years of age, we also compared copper liver tissue to the HII after removing patients < 13 years of age. Again, there was no significant correlation between liver copper concentration and HII (Pearson correlation coefficient = 0.040; p=0.62). However, four patients in this study population had an iron concentration > 3000 mcg/g which is indicative of iron overload. Additionally, 13 patients aged 13 or older in this sample population had a HII > 1.0 and three of these patients had a HII > 1.9 which may indicate secondary iron overload.

When iron liver tissue concentrations were stratified by gender, the median iron concentration was significantly higher (p<0.001) in males (387.5 mcg/g, IQR=232.3-849.5) vs females (196.0 mcg/g, IQR=118.0-597.5)). Furthermore, as a function of age and gender (Fig. 2), iron levels increase in the liver in males with age (Pearson correlation coefficient 0.231; p=0.017), however the correlation is not as strong in females (Pearson correlation coefficient = 0.094; p=0.38). To avoid selection bias, we did not compare copper liver tissue values by gender or test by gender and age, since patients were selected based on their copper values.

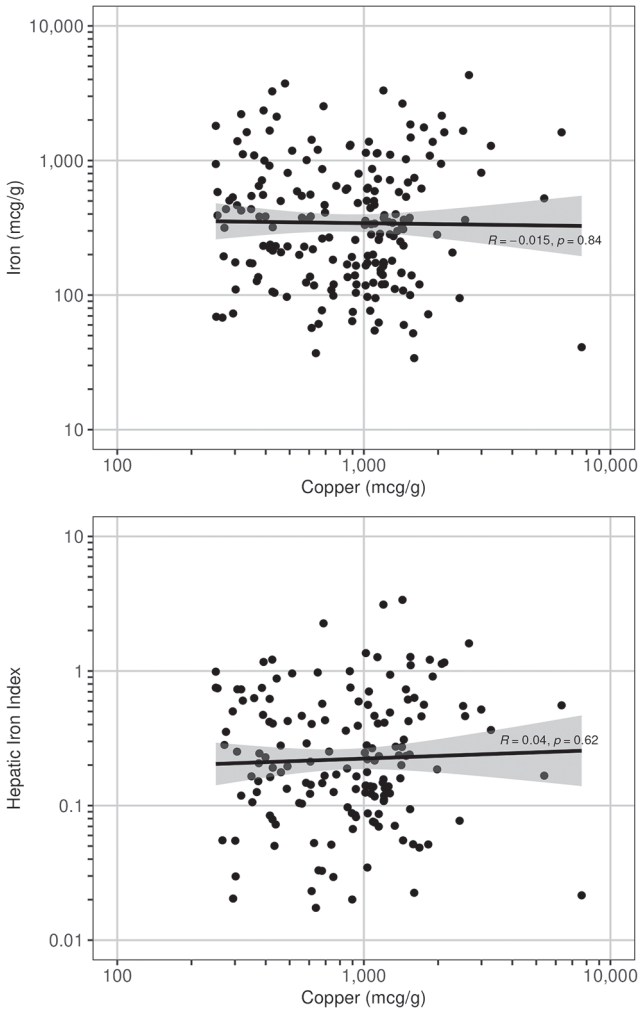

Fig. 1. (Top) Copper liver biopsy concentration vs iron liver biopsy concentration. (Bottom) Copper liver biopsy concentration vs hepatic iron index.

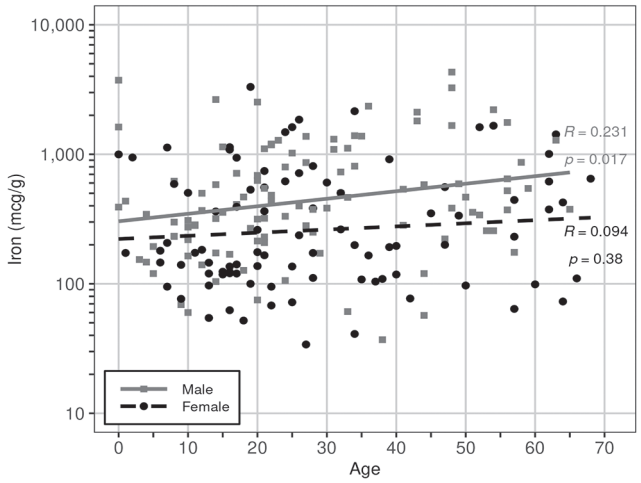

Fig. 2. Age vs iron liver biopsy concentration in males and females.

Next, we analyzed median iron concentration by copper level, dividing the population into 'suggestive of WD' (250-1000 mcg/g) and 'strongly suggestive of WD' (≥ 1000 mcg/g), and found no significant difference (376.0 vs 338.0, p=0.92). Interestingly, (Fig. 3) although median iron concentration was not significantly different between the groups, 10 patients (13.2%) had a HII >1 in the ≥ 1000 mcg/g copper group, whereas only 3 (3.7%) had a HII greater that 1 in the 250-1000 mcg/g copper group (p=0.041).

Confirmed WD Population

Ten patients had a confirmed clinical diagnosis of WD. Clinical characteristics of these patients are summarized in Table I. The median age of the patient at time of liver biopsy was 21.5 years (range 16.75-33.25). Six (60%) liver biopsy samples were from females and four (40%) liver biopsy samples were from males. Three (30%) of these patients had a liver transplant.

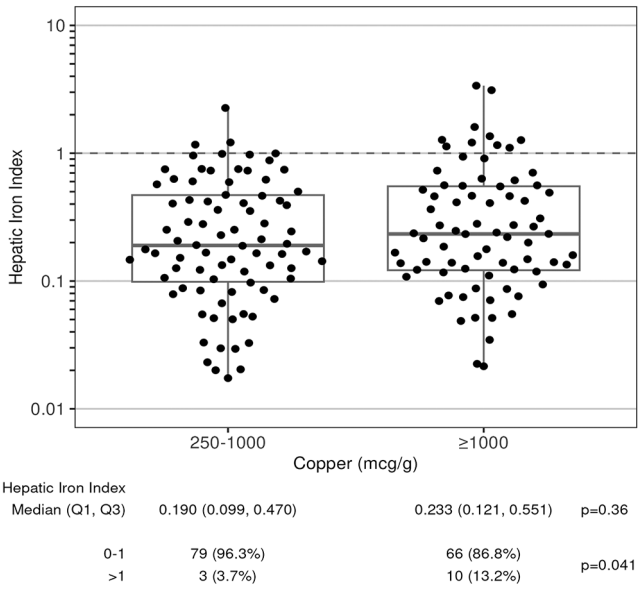

Fig. 3. Boxplots of hepatic iron index values in patients with a copper liver biopsy concentration between 250-1000 mcg/g and with copper liver biopsy concentrations ≥ 1000 mcg/g.

Of the ten patients with WD, six had a liver copper concentration between 250-1000 mcg/g. The remaining four patients had a liver tissue copper concentration of ≥ 1000 mcg/g. As is shown in Fig. 4, the median liver iron concentration was 1386 mcg/g in patients with ≥ 1000 mcg/g copper and was 470 mcg/g in patients with 250-1000 mcg/g copper (p=0.29). Furthermore, the three patients who went on to have a liver transplant had some of the highest copper and iron liver biopsy concentrations within this population.

Four of the patients with WD had ferritin measured. Three patients had a liver copper concentration of 250-1000 mcg/g and ferritin values of 65, 169, and 442 mcg/L. One patient with a copper liver biopsy value of ≥ 1000 mcg/g had a ferritin of 2 mcg/L which is below the reference range.

Varying numbers of patients had ALT, AST, AFP and ALP measured during the clinical WD workup. Five out of seven patients with available ALT and AST lab values had elevated ALT and AST values. Two patients had an AFP ordered and both had elevated AFP values. Of six patients with available ALP lab values, one had elevated ALP. Of note, the patient with the highest ALT and AST values went on to have a liver transplant.

Table 1. Characteristics of 10 confirmed Wilson disease (WD) patients

Patient#	Age at Diagnosis	Duration of Disease prior to Liver Biopsy	Biopsy for Diagnosis or Monitoring	Blood Transfusions prior to Biopsy	Genetic Testing Performed	Liver Biopsy Result	Hepatic Copper Content
1	18	50 years	Monitoring	Multiple RBC transfusion >1 month after biopsy date	No	Cirrhosis with moderately active chronic hepatitis, consistent with WD.	773 mcg/g of dry weight
2	16	~1 year	Diagnosis	No	Yes	Panacinar hepatitis, mild steatosis, and WD	increased hepatic copper consistent with v
3	6	15 years	Monitoring	No	Yes	Bridging fibrosis with histologic features consistent with patient's diagnosis of WD	882 mcg/g of dry weight
4	12	N/A	Diagnosis	No	Yes	Mildly active (grade 1 of 3) steatohepatitis and elevated hepatic copper content	749 mcg/g of dry weight
5	21	N/A	Monitoring	No	No	Histologic features of WD, including 30% macrovesicular steatosis, conspicuous ballooning hepatocytes, and increased periportal copper deposition	276 mcg/g of dry weight
6	10	<1	Diagnosis	No	Yes	Mild nonspecific portal inflammation with focal ductal and ductular proliferation; patchy mild portal fibrosis and elevated hepatic copper content	285 mcg/g of dry weight
7	7	12	Monitoring	No	Yes	Hepatic parenchyma with rare macrovesicular steatosis (<5%).	1278 mcg/g of dry weight
8	Related to WD, but not confirmed	N/A	Monitoring	No	Yes	Inactive cirrhosis, morphologically cryptogenic	7641 mcg/g of dry weight
9	54	N/A	Diagnosis	Yes, post liver transplant	Yes	Severe active steatohepatitis with bridging fibrosis and very elevated hepatic copper content suggestive of WD.	2526 mcg/g of dry weight
10	19	N/A	Diagnosis	Yes, post liver transplant	Yes	Established cirrhosis (stage 4 of 4) with marked copper deposition, confluent areas of hepatocyte necrosis, and features of steatohepatitis, suggestive of WD	1200 mcg/g of dry weight

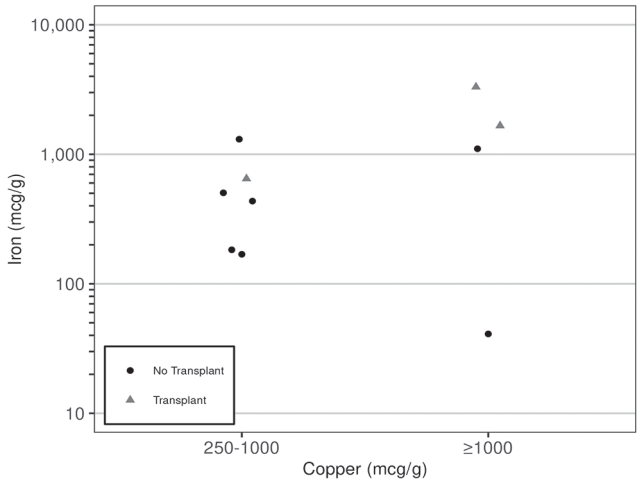

Fig. 1. Iron liver biopsy concentrations in clinically confirmed WD patients with a copper liver biopsy between 250-1000 mcg/g and ≥ 1000 mcg/g.

Eight of ten patients with WD had a bilirubin result available. As shown in Fig. 5, of the five patients with a copper liver concentration between 250-1000 mcg/g, all 5 had normal bilirubin. Of the three patients with a copper liver concentration ≥ 1000 mcg/g, all three had elevated bilirubin levels. It should also be noted that the two patients with the highest bilirubin levels went on to have a liver transplant.

Eight patients with WD had red blood cell, Hb and hematocrit values available in the medical record. Three of eight WD patients had low RBC values. As shown in Fig. 6, the two patients with copper ≥ 1000 mcg/g had Hb measured and exhibited low Hb for their age and sex, while all six patients with a copper between 250-1000 mcg/g had normal Hb values (p=0.036). The same relationship held true for hematocrit (p=0.036).

DISCUSSION

In our population of 197 liver biopsy samples with a copper concentration ≥ 250 mcg/g, there was no significant association between copper and iron levels in the liver. However, as shown in Fig. 3, 8% had a HII > 1.0. Additionally, in our subset of patients confirmed to have WD, one patient had a HII greater than 1.9 which is indicative of iron overload [12]. These findings are consistent with previous reports by other groups that have identified iron overload in WD patients [8, 17, 18]. As recommended by Pak et al. [8], it may be appropriate to monitor iron indices in patients with WD on a regular basis. Furthermore, treating physicians should also consider ordering liver iron levels at the same time as liver copper levels to assess for iron overload.

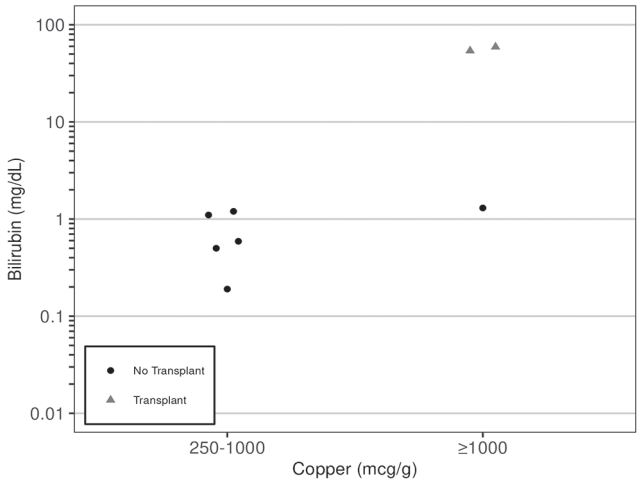

Fig. 5. Bilirubin concentrations in clinically confirmed WD patients with a copper liver biopsy between 250-1000 mcg/g and ≥ 1000 mcg/g.

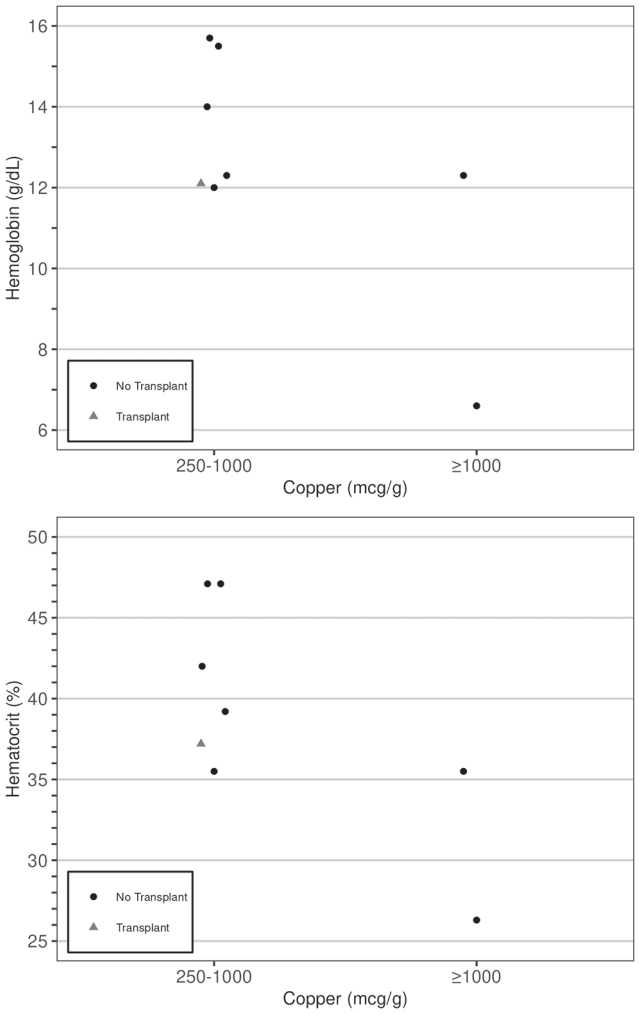

Fig. 6. (Top) Hemoglobin concentrations in clinically confirmed WD patients with a copper liver biopsy between 250-1000 mcg/g and ≥ 1000 mcg/g. (Bottom) Hematocrit values in clinically confirmed WD patients with a copper liver biopsy between 250-1000 mcg/g and ≥ 1000 mcg/g.

In the 197 liver biopsy samples, males had significantly higher liver iron 387.5 mcg/g (232.3-849.5) than females 196.0 mcg/g (118.0-597.5), p<0.001. Though not statistically significant, this same trend appears to be true in the confirmed WD subset. Males present with iron overload more often than females and have a higher incidence of liver injury [19]. Additionally, it is thought that women may have lower iron concentrations due to blood loss experienced with menstruation [19]. Due to selection bias concerns, we could not determine whether gender differences also existed related

to copper liver tissue concentrations in this population. Future research into iron and copper liver tissue concentrations and rates of liver failure as relates to gender should be investigated.

When evaluating bilirubin and liver copper concentrations, there appears to be an association between elevated liver copper concentrations and elevated bilirubin concentrations (Fig. 5). As bilirubin is used to assess liver function, it may be a suitable indicator of liver failure in WD patients. The two patients with the highest bilirubin levels had the highest liver copper values. This potential association should be further studied in a much larger population of clinically diagnosed WD patients without and without liver transplant.

Perhaps the most interesting findings from the WD diagnosed patient population was the negative association between Hb and hematocrit and liver copper concentrations. Patients with a copper liver tissue concentration > 1000 mcg/g seemed to have significantly lower hematocrit and Hb in our clinically diagnosed WD population. Hemolytic anemia has been documented in patients with WD [20-22]. Additionally, anemia in patients with copper deficiency has also been documented [23]. Furthermore, severe hemolysis and hepatic decompensation can occur together in WD which can be fatal if not treated [24]. Interestingly, in one study by Attri et al. [21], elevated levels of non-ceruloplasmin copper were found in all WD patients during episodes of hemolytic anemia [21]. Thus, it is an intriguing possibility that monitoring of hemoglobin and/or hematocrit could be an easy laboratory assay to monitor the progression of WD as well as potential liver decompensation in patients with WD and as a possible indication for liver transplant.

There are several limitations to our study. First, for the primary dataset of 197 copper and iron liver tissue biopsies, our group did not have access to the clinical information or medical records for every sample. While having a copper liver tissue concentration of > 250 mcg/g is suggestive of WD and a copper liver tissue of ≥ 1000 mcg/g is strongly suggestive of WD, it is certainly not a standalone diagnostic test of WD. Therefore, these liver tissue biopsy samples could be from potential confounding cholestatic liver diseases such as primary sclerosing cholangitis and primary biliary cirrhosis [2]. When evaluating data from the subset of patients with a confirmed clinical diagnosis of WD (n=10), caution should be taken when considering correlations associated with the data as the total number of patients is very small. As WD is a relatively rare disorder, these same analyses should be further investigated at institutions that are WD "centers of excellence" where the number of patients with confirmed WD is more plentiful.

CONCLUSION

Our findings suggest that secondary iron overload may occur in patients with liver biopsy copper concentrations suggestive of WD. In this study population, 8% of patients with a liver biopsy copper concentrations > 250 mcg/g dry weight had a HII greater than 1. Furthermore, one out of ten patients (10%) in our analysis with confirmed diagnosis of WD had a hepatic iron index greater than 1.9. These findings may warrant clinicians to regularly monitor not only copper and copper binding proteins, such as ceruloplasmin, in patients with WD, but also regularly monitor iron indices as well. Iron levels may be important prognostic indicators in WD, however more research is required to determine its utility.

Furthermore, the analysis of various common laboratory values in patients with confirmed WD indicated bilirubin, Hb and hematocrit may be associated with liver copper biopsy concentrations and may indicate the outcome of liver transplant. The sample size of this population, however, is relatively small and should be confirmed in a larger WD population.

Conflicts of interest: None to declare.

Authors' contribution: P.L.D conceived the study. All the authors contributed to the designed of the study. P.L.D., R.F.F., P.V. collected data. G.S. analyzed the data. P.L.D., R.F.F., P.V., G.S. drafted the manuscript. K.W., J.B., S.H. and P.J.J. revised the manuscript for important intellectual content. P.J.J. is the guarantor of this study.

Supplementary material: To access the supplementary material visit the online version of the *J Gastrointestin Liver Dis* at http://dx.doi.org/10.15403/jgld-5671.

REFERENCES

1. Roberts EA, Schilsky ML. Current and Emerging Issues in Wilson's Disease. N Engl J Med 2023;389:922-938. doi:10.1056/NEJMra1903585
2. Schilsky ML. Wilson disease: Clinical manifestations, diagnosis and natural history. In: UpToDate, Rand EB, Aminoff MJ, Runyon B. (Eds.), Wolters Kluwer. Accessed on January 5, 2023.
3. Bitzer AC, Fox J, Day PL, et al. Establishment of a Labile Bound Copper Reference Interval in a Healthy Population via an Inductively Coupled Plasma Mass Spectrometry Dual Filtration-Based Assay. Arch Pathol Lab Med 2024;148:818-827. doi:10.5858/arpa.2023-0259-OA
4. Catana AM, Medici V. Liver transplantation for Wilson disease. World J Hepatol 2012;4:5-10. doi:10.4254/wjh.v4.i1.5
5. Ferri FF. Ferri's Clinical Advisor 2024. 1st ed. St. Louis; Elsevier. 2023.
6. Copper, Liver Tissue. Mayo Clinic Laboratories Test Catalog. Available from: https://www.mayocliniclabs.com/test-catalog/overview/8687#Clinical-and-Interpretive
7. Vasilyev VB. Looking for a partner: ceruloplasmin in protein-protein interactions. Biometals 2019;32:195-210. doi:10.1007/s10534-019-00189-1
8. Pak K, Ordway S, Sadowski B, Canevari M, Torres D. Wilson's Disease and Iron Overload: Pathophysiology and Therapeutic Implications. Clin Liver Dis (Hoboken) 2021;17:61-66. doi:10.1002/cld.986
9. Hsu CC, Senussi NH, Fertrin KY, Kowdley KV. Iron overload disorders. Hepatol Commun 2022;6:1842-1854. doi:10.1002/hep4.2012
10. Brissot P, Pietrangelo A, Adams PC, de Graaff B, McLaren CE, Loréal O. Haemochromatosis. Nat Rev Dis Primers 2018;4:18016. doi:10.1038/nrdp.2018.16
11. Batts KP. Iron overload syndromes and the liver. Mod Pathol 2007;20 Suppl 1:S31-S39. doi:10.1038/modpathol.3800715
12. Summers KM, Halliday JW, Powell LW. Identification of homozygous hemochromatosis subjects by measurement of hepatic iron index. Hepatology 1990;12:20-25. doi:10.1002/hep.1840120105

13. Bermejo F, García-López S. A guide to diagnosis of iron deficiency and iron deficiency anemia in digestive diseases. World J Gastroenterol 2009;15:4638-4643. doi:10.3748/wjg.15.4638
14. Camaschella C, Poggiali E. Towards explaining "unexplained hyperferritinemia". Haematologica 2009;94:307-309. doi:10.3324/haematol.2008.005405
15. Burtis CA, Bruns DE. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 7th ed. St. Louis: Elsevier; 2015.
16. Jirun P, Zhang G, Kim HK, et al. Clinical utility of alpha fetoprotein and HCCR-1, alone or in combination, in patients with chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Dis Markers 2011;30:307-315. doi:10.3233/dma-2011-0789
17. Shiono Y, Wakusawa S, Hayashi H, et al. Iron accumulation in the liver of male patients with Wilson's disease. Am J Gastroenterol 2001;96:3147-3151. doi:10.1111/j.1572-0241.2001.05269.x
18. Hayashi H, Yano M, Fujita Y, Wakusawa S. Compound overload of copper and iron in patients with Wilson's disease. Med Mol Morphol 2006;39:121-126. doi:10.1007/s00795-006-0326-7
19. Harrison-Findik DD. Gender-related variations in iron metabolism and liver diseases. World J Hepatol 2010;2:302-310. doi:10.4254/wjh.v2.i8.302
20. Sharma S, Toppo A, Rath B, Harbhajanka A, Lalita Jyotsna P. Hemolytic Anemia as a Presenting Feature of Wilson's Disease: A Case Report. Indian J Hematol Blood Transfus 2010;26:101-102. doi:10.1007/s12288-010-0034-2
21. Attri S, Sharma N, Jahagirdar S, Thapa BR, Prasad R. Erythrocyte metabolism and antioxidant status of patients with Wilson disease with hemolytic anemia. Pediatr Res 2006;59:593-597. doi:10.1203/01.pdr.0000203098.77573.39
22. Ye XN, Mao LP, Lou YJ, Tong HY. Hemolytic anemia as first presentation of Wilson's disease with uncommon ATP7B mutation. Int J Clin Exp Med 2015;8:4708-4711.
23. Wazir SM, Ghobrial I. Copper deficiency, a new triad: anemia, leucopenia, and myeloneuropathy. J Community Hosp Intern Med Perspect 2017;7:265-268. doi:10.1080/20009666.2017.1351289
24. Iser JH, Stevens BJ, Stening GF, Hurley TH, Smallwood RA. Hemolytic Anemia of Wilson's Disease. Gastroenterology 1974;67:290-293. doi:10.1016/S0016-5085(19)32893-8