

Copper Deficiency in Liver Diseases: A Case Series and Pathophysiological Considerations

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Copper is an indispensable trace element. It serves as a cofactor for enzymes involved in cellular energy metabolism, antioxidant defense, iron transport, and fibrogenesis. Although these processes are central in the pathogenesis of liver disorders, few studies have attributed them to copper deficiency. We herein describe in detail a case series of liver disease patients (n = 12) who presented with signs of copper deficiency based on serum and liver copper measurements. Median age of the group at the time of presentation was 39 (range 18-64 years). Six patients were female. The median serum copper was 46 µg/dL (normal range: 80-155 µg/dL for women and 70-140 µg/dL for men). Seven of the 12 patients had hepatic copper concentration less than 10 µg/g dry weight (normal range: 10-35 µg/g). Most cases presented with acute-on-chronic liver failure (n = 4) and decompensated cirrhosis (n = 5). Only 3 patients had a condition known to be associated with copper deficiency (ileocolonic Crohn's disease following resection n = 1, Roux-en-Y gastric bypass n = 2) before presenting with hepatic dysfunction. Notable clinical features included steatohepatitis, iron overload, malnutrition, and recurrent infections. In 2 of the 3 patients who received copper supplementation, there was an improvement in serum copper, ceruloplasmin, and liver function parameters. **Conclusion:** Copper deficiency in the serum or liver occurs in a wide range of liver diseases. Given the biological essentiality of copper, the mechanism and clinical significance of this association require systematic study. (*Hepatology Communications* 2019;3:1159-1165).

The role of copper in liver disorders is best recognized in Wilson's disease, in which hepatic copper accumulation is not only pathogenic, but also pathogenic as a source of cellular reactive oxygen species.⁽¹⁾ Elevated hepatic copper is also found in cholestatic liver diseases, but this is likely a consequence of decreased biliary excretion of copper and not a cause of the underlying liver disease.^(2,3) The opposite end of the spectrum (reduced hepatic copper concentration) is rarely reported in patients with liver diseases.⁽⁴⁾ In mammals, cytochrome-c oxidase, ceruloplasmin, hephaestin, and

copper-zinc superoxide dismutase represent a partial list of cuproenzymes whose metabolism and function depend on copper availability.⁽⁵⁻⁸⁾ In addition, copper plays a key role in the innate immune system, acting as a "bullet" for effective killing of bacteria and fungi by macrophages.⁽⁹⁾ Consequences of copper deficiency include iron overload, tissue fibrosis, cytopenia, and susceptibility to infections.⁽¹⁰⁻¹⁴⁾ Although these clinical features are also prevalent in advanced cirrhosis,⁽¹⁵⁻¹⁷⁾ the significance of copper deficiency in liver disorders has only been reported in human fatty liver disease and preclinical animal models.⁽¹⁸⁻²⁰⁾

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We herein describe 12 patients who presented with copper deficiency in the setting of liver dysfunction, primarily in the form of advanced cirrhosis and acute-on-chronic liver failure. Based on our current understanding of the role of copper in mammalian physiology, we discuss the clinical implication of copper deficiency in the development and progression of liver disease.

Case Series

CASE ASCERTAINMENT

All 12 patients were receiving routine clinical care for liver disease presentations at the University of Washington Medical Center between 2010 and 2017. The patients were seen by two of the authors (L.Y. and I.W.L.) and noted for their low serum and hepatic copper measurements. Clinical variables were extracted retrospectively. No consents were required according to our institutional review board, except in case 8, who provided consent to additional tissue analysis. Serum copper and ceruloplasmin concentrations were measured to assess micronutrient status and Wilson's disease. Hepatic copper concentration was measured to evaluate for Wilson's disease. Both serum and hepatic copper concentrations were measured with cell-inductively coupled plasma-mass spectrometry. Serum ceruloplasmin was measured using nephelometry. Liver specimens were fixed in formalin and embedded in paraffin. Hepatic copper concentration was expressed as micrograms per gram of dry weight of the specimen. The median time between serum and hepatic copper concentration measurements was 8 days (range 0-70 days). Serum biochemical tests were performed at the University of Washington. Hepatic

and urinary copper measurements were performed by the Mayo Medical Laboratories.

LIVER TISSUE STUDY AND CORRELATION WITH SERUM COPPER

Liver histology showed cirrhosis in most (n = 7). Steatohepatitis (n = 5) and iron overload (n = 3) were other prominent features (Fig. 1 and Table 1). Case 3's biopsy showed 40%-50% simple steatosis without fibrosis or inflammation. Case 8's biopsy showed more extensive steatosis (80%) with mild pericellular fibrosis. In cases 2, 3, 6 and 12, even though serum copper concentrations were low (19-63 µg/dL), hepatic copper concentrations were normal, and in case 12, mildly elevated (17-77 µg/g). In seven cases, low serum copper correlated with below normal hepatic copper concentrations. There is also a strong correlation between serum copper and serum ceruloplasmin concentrations (Pearson correlation coefficient = 0.799). Compared with patients with normal hepatic copper, patients with low hepatic copper concentrations were older, more likely to be female, and more likely to have known risk factors for copper deficiency. These differences, however, did not achieve statistical significance (Table 2). In case 8, with the patient's informed consent and assistance from a laboratory dedicated to trace metal analysis, hepatic copper concentrations from three segments of the liver explant all showed a concentration below 5 µg/g dry weight.

BASELINE CLINICAL CHARACTERISTICS

Key characteristics and additional details of 12 patients according to age of presentation are provided

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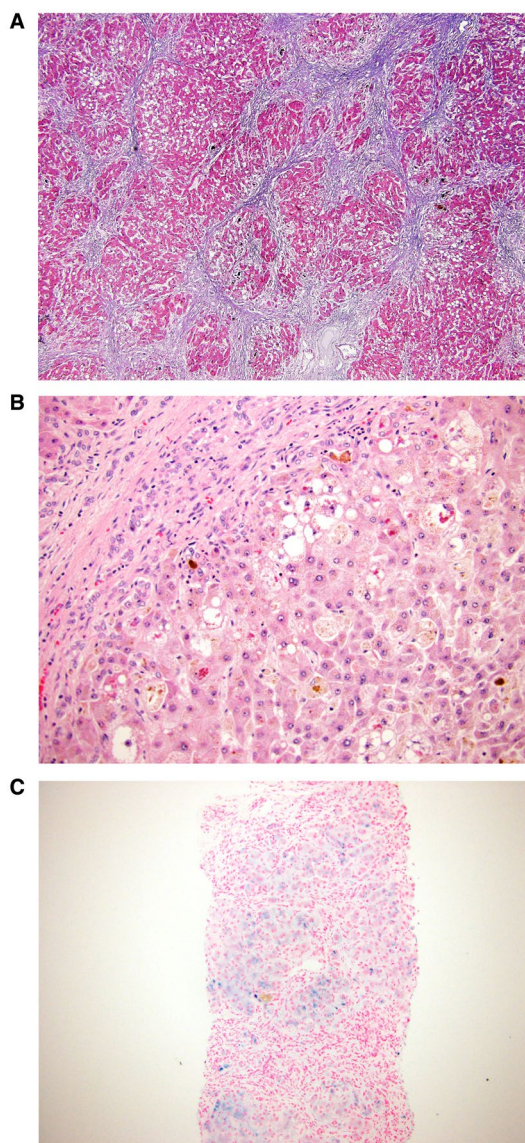


FIG. 1. Representative liver histology of liver disease patients with copper deficiency. (A) Cirrhotic liver parenchyma (case 8), Masson's trichrome stain $\times 100$. (B) Steatohepatitis with Mallory Denk bodies and cholestasis (case 8), hematoxylin and eosin stain $\times 200$. (C) Moderate iron overload (case 5), Prussian blue stain $\times 20$.

in Table 1 and Supporting Table S1, respectively. The median age of the group at the time of presentation was 39 (range 18–64 years). Six were female. All subjects except case 1 were Caucasian. Only cases 3 and 7 did not have features of severe liver disease. Case 3 presented for evaluation of elevated liver enzymes (2 to 5 times upper limit of normal, ranging between 80 and 200 units/L during follow-up). Case 7 had

intermittently elevated liver enzymes (ranging from normal to 1.5 times upper limit of normal).

All cases except 1, 3, and 7 presented with at least one clinical feature that has been associated with copper deficiency (Supporting Table S1). These included ataxia, iron overload with elevated transferrin saturation or ferritin, unexplained and transfusion dependent anemia, or recurrent bacterial infections (≥ 2 episodes in 3 months). Cases 5, 6, and 10 had normocytic anemia with hemoglobin ranging between 6 and 8 g/dL before transfusions. None had overt signs of bleeding from the gastrointestinal tract. Cases 5 and 6 had small nonbleeding esophageal varices and minimal portal hypertensive gastropathy. Case 10's upper endoscopy was normal. None of these 3 patients had signs of iron deficiency or hemolysis based on serum studies. Cases 6 and 10 also underwent bone marrow biopsy that did not show signs of primary hematological malignancies. Even though case 10's serum studies did not show signs of iron deficiency, her marrow showed minimal iron stores. Her anemia persisted despite temporary iron infusion.

All but 2 patients (cases 3 and 7) had serum albumin below 3 g/dL. Malnutrition based on subjective global assessment as well as nutritionist records was present in all except cases 1, 3, and 7. Two patients, cases 4 and 10, had a history of Roux-en-Y gastric bypass—a condition known to be associated with copper deficiency.⁽²¹⁾ Case 4, whose bypass surgery was 18 months before her presentation, had overt diarrhea before presenting with hepatic decompensation. Case 2 used zinc sulfate supplementation (220 mg daily) for 6 months before his presentation. At the time of his liver biopsy, his serum zinc remained low (49 $\mu\text{g/dL}$). No patients were treated with parenteral nutrition at the time of their serological or hepatic copper assessment. Three patients had spot urinary protein greater than 100 mg/dL (range 300–600 mg/dL) (Supporting Table S1). These elevated measurements were in the setting of acute tubular injury (cases 8 and 10) and urosepsis (case 9). Urine analysis also showed significantly elevated red blood cells (cases 8 and 10), white blood cells (case 9), and casts.

CLINICAL OUTCOME AND COPPER SUPPLEMENTATION

Cases 3 and 7, who presented with abnormal liver enzymes, had a benign clinical course without signs

TABLE 1. SUMMARY OF KEY CLINICAL CHARACTERISTICS IN 12 PATIENTS WHO PRESENTED WITH COPPER DEFICIENCY AND LIVER DISEASES

Case	Age	Sex	Liver Disease Presentation	Serum Copper ($\mu\text{g/dL}$)*	Hepatic Copper ($\mu\text{g/g}$) [†]	Serum Cp (mg/dL) [‡]	Serum Zinc ($\mu\text{g/dL}$) [§]	Serum Ferritin (ng/mL)	Histology	Clinical Outcome	Duration of Follow-up (Months)
1	18	M	Acute liver failure of unknown etiology	71	<10	24	NA	3,543	Explant: Necrosis, minimal fibrosis	Transplantation, alive and well	60
2	27	M	Decompensated cirrhosis, HCV	19	26	8	54	172	Biopsy: Cirrhosis	Died	40
3	31	M	Abnormal liver enzymes	50	38	22	87	347	Biopsy: Minimal fibrosis, steatosis	Asymptomatic, alive and well	102
4	34	F	Acute-on-chronic liver failure, obesity	41	NA	11	38	1,644	Biopsy: Bridging fibrosis, steatohepatitis	Full recovery after parental nutrition, IV and oral copper	34
5	35	F	Acute-on-chronic liver failure, alcohol	NA	<10	NA	51	1,698	Biopsy: Cirrhosis, no steatosis, moderate iron	Died from multiorgan failure	3
6	37	F	Decompensated cirrhosis, alcohol	63	17	16	32	2,129	Biopsy: Cirrhosis, no steatosis, moderate iron	Died	13
7	41	M	Abnormal liver enzymes	60	<10	16	71	191	Biopsy: minimal fibrosis, steatosis, mild iron	Alive and well	12
8	41	M	Decompensated cirrhosis, obesity	37	<10	10	NA	159	Explant: Cirrhosis, steatohepatitis	Transplantation, alive, persistent kidney failure	45
9	43	F	Acute-on-chronic liver failure	46	<10	9	27	773	Explant: Cirrhosis, steatohepatitis	Transplantation, alive and well	47
10	44	F	Acute-on-chronic liver failure, obesity	36	<10	9	33	473	Biopsy: Bridging fibrosis, steatohepatitis	Died from multi-organ failure	1
11	58	F	Decompensated cirrhosis	58	<10	16	37	40	Biopsy: Cirrhosis, no steatosis	Treated with oral nutrition, copper supplement, full recovery of liver function	57
12	64	M	Decompensated cirrhosis, HBV, and HCC	42	77	NA	28	1,728	Explant: Cirrhosis, severe iron overload	Died after liver transplantation	5

*Normal serum copper: 80–155 $\mu\text{g/dL}$ for women and 70–140 $\mu\text{g/dL}$ for men.

[†]Normal hepatic copper concentration: 10–35 $\mu\text{g/g}$ dry weight.

[‡]Normal serum ceruloplasmin (Cp): 22.0–66.0 mg/dL.

[§]Normal serum zinc: 60–120 $\mu\text{g/dL}$.

^{||}Normal serum ferritin: 10–180 ng/mL.

Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IV, intravenous; and NA, not available.

TABLE 2. COMPARISON BETWEEN PATIENTS WITH LOW AND NORMAL HEPATIC COPPER CONCENTRATIONS

	Hepatic copper ($\mu\text{g/g}$ dry weight)		P-value
	>10	<10	
N	4	7	-
Age (median)	34	41	0.6
Sex (N, % female)	1 (25%)	4 (57%)	0.3
Having known risk factor for copper deficiency (N, %)	0	2 (28%)	0.3
Histologically confirmed cirrhosis (N, %)	3 (75%)	4 (57%)	0.6
Serum copper (median, $\mu\text{g/dL}$)	46	52	0.7
Serum ceruloplasmin (median, mg/dL)	16	13	0.8
Serum zinc (median, $\mu\text{g/dL}$)	43	37	0.8
Malnutrition present (N, %)	3 (75%)	5 (71%)	0.9
Deaths (N, %)	3 (75%)	2 (28%)	0.1

of hepatic decompensation during follow-up. In the other 10 patients who presented with acute liver failure ($n = 1$), acute-on-chronic liver failure ($n = 4$) or decompensated cirrhosis ($n = 5$), mortality rate was 50% ($n = 5$) and transplantation rate was 40% ($n = 4$) over a median follow-up of 37 months. The only mortality after liver transplant was subject 12, who died 5 months after surgery from liver cancer recurrence and heart failure.

Cases 4 and 11 recovered following intensive nutritional support, including copper supplementation, with subsequent normalization of hepatic synthetic function. Case 4, who underwent Roux-en-Y gastric bypass surgery 18 months before presentation, was supplemented with IV copper, 2 mg per day, as part of her parenteral nutrition for 3 months. Her serum copper was not repeated, but her serum ceruloplasmin improved from 11 mg/dL at initial presentation to 17 mg/dL after supplementation. Her liver function normalized and remained stable 3 months after parenteral nutrition was stopped. Case 11, who had inactive ileocolonic Crohn's disease and distant history of ileum and right colon resection, was supplemented with oral copper 2 mg daily for 1 year with improvement of her serum copper (41 to 94 $\mu\text{g/dL}$), ceruloplasmin (16 to 23 mg/dL), and albumin concentrations (1.9 to 3.5 g/dL). In case 10, who presented with acute-on-chronic liver failure 12 years after Roux-en-Y gastric bypass, oral copper 2 mg over a 3-week period improved serum

copper from 36 to 60 $\mu\text{g/dL}$. The patient, however, died of fungal sepsis and multi-organ failure. Copper was not supplemented in any other cases.

Discussion

Likely prompted by its dramatic elevation in the liver parenchyma in Wilson's disease, copper has long been an interest in hepatology. Early investigations failed to find low hepatic copper concentrations in a wide range of liver diseases.⁽²²⁻²⁵⁾ In South Asia, serum copper concentrations were consistently higher in patients with cirrhosis than healthy controls, and were higher in more advanced cirrhosis according to Child-Pugh class.^(26,27) To our knowledge, Thackery et al. described the only series in which 4 patients with myeloneuropathy in the setting of compensated liver disease had low serum copper concentrations—the traditional definition of copper deficiency.^(4,28,29) Only 1 patient had low hepatic copper concentration.⁽⁴⁾ The current case series is therefore the largest to date that documents copper deficiency in the serum and liver tissue in patients with advanced liver disease.

Because copper in the blood is mostly bound to ceruloplasmin and to a lesser extent albumin and other amino acids,^(30,31) one plausible explanation for reduced serum copper concentrations in our patients is protein malnutrition commonly found in cirrhosis.⁽³²⁾ Unlike albumin, however, serum ceruloplasmin concentrations are normal in most patients with advanced liver disease.⁽³³⁻³⁵⁾ We therefore suspect that protein malnutrition, even in the setting of cirrhosis with reduced hepatic synthetic function, may not be the only explanation for reduced serum copper concentrations in liver disease. It is conceivable that reduced whole-body copper store had caused a decrease in serum ceruloplasmin, which has a high rate of turnover when copper is not available.⁽⁶⁾ Documented improvement of serum ceruloplasmin following copper supplementation in cases 4 and 11 corroborates with this suggestion. Other considerations include redistribution of copper from serum to body compartments other than the liver. For example, musculoskeletal copper may account for up to 50% of the total body copper pool.⁽³⁶⁾ Finally, mutation in the ceruloplasmin gene has been associated with hypocupremia, hemosiderosis of the liver, and neurological complaints.⁽³⁷⁾ To the best of our knowledge, whether ceruloplasmin gene mutation can manifest as advanced

liver disease is unknown. Future research should assess copper content in other body compartments, ceruloplasmin gene mutation, as well as whether copper-carrying capacity by ceruloplasmin is affected by liver diseases.

Low serum copper was associated with low hepatic copper in approximately half of the patients in our series. Because zinc availability affects the synthesis of metallothionein, the main copper storage protein in the liver,⁽³⁸⁾ we suspected that patients with low hepatic copper might have lower serum zinc concentrations. However, there was no statistically significant difference in serum zinc between patients with low or normal hepatic copper. Because zinc level is reduced in most cases, our small series is likely underpowered to detect a significant difference. It is possible that copper deficiency in cirrhosis is a continuum in which the “low serum–low hepatic copper” group has more severe deficiency compared with the “low serum–normal hepatic copper” group.

Reduced copper availability affects iron homeostasis, because ferroxidase activity of ceruloplasmin (in the plasma) and hephaestin (at the brush border of small intestine) depend on copper as a cofactor.^(7,39–41) The current series therefore implicates a potential role of copper deficiency in two common hepatic disorders in which perturbation in iron homeostasis are well recognized. The first disorder is fatty liver disease, in which lower serum and hepatic copper are associated with hepatic iron accumulation and more advanced steatosis.⁽¹⁸⁾ The degree of copper deficiency, however, is more pronounced in the current series. The second disorder is acute-on-chronic liver failure, in which an elevated serum labile iron pool are associated with an increased mortality.⁽⁴²⁾ In alcoholic hepatitis, the most common form of acute-on-chronic liver failure, iron plays an important role in producing a chemoattractant for neutrophils.⁽⁴³⁾ Interestingly, despite an increased inflammatory response in the liver tissue and peripheral blood, neutrophils in alcoholic hepatitis do not function properly and this abnormality is associated with an elevated infection risk and organ failure.⁽⁴⁴⁾ To our knowledge, no studies in acute-on-chronic liver failure have assessed whether changes in iron homeostasis and neutrophils (whose maturation and function depend on copper) is related to copper deficiency.^(45,46) This may be important, because if copper deficiency indeed exists, it becomes a potential target of intervention in which copper supplementation might (1) improve pathological consequences of

iron overload^(47,48) and (2) restore immune cell function as it did in experimental animals.⁽¹⁴⁾

In summary, our series substantiates the connection between liver disease and copper deficiency. We also provided evidence that copper supplementation was a safe and effective adjunct in certain cases of hepatic decompensation. Because these patients represent a selected cohort who underwent routine clinical care, it was not possible for us to define the mechanism of hypocupremia, its prevalence, or its impact on the natural history of liver disease manifestations. Understanding the nature of copper deficiency in liver disease, as in zinc and selenium,^(49,50) will require a larger and unbiased patient population. Based on the current evidence, we believe it would be reasonable to screen for copper deficiency in patients with liver dysfunction who have additional risk factors (for copper deficiency) or who are significantly malnourished. Oral or parenteral supplementation should be considered with close monitoring.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep4.1393/supinfo.