

Ceruloplasmin Activity and Ferritin in Patients with Chronic Liver Disease

Mohammed Hussein Habeeb, Mufeed Jalil Ewadh, Mazen Jaafer Mousa

College of Medicine, University of Babylon, Babylon, Hillah, Iraq

Abstract

Background: Ceruloplasmin (CP) is a copper-containing glycoprotein found in the globulin portion of human blood serum. CP, a glycoprotein secreted by hepatocytes, carries more than 90% of copper in the bloodstream of healthy people. CP is an effective antioxidant that prevents lipid peroxidation by removing oxygen. CP inhibits lipid peroxidation initiation. CP has function also known as an extracellular superoxide dismutase species (SOD) that neutralizes radicals of superoxide anions. **Aims:** The aim of this study is to understand the status of CP in chronic liver disease (CLD) patients, which could yield important information regarding the overall oxidative stress and iron storage. Out of the total 86 samples, 50 samples were patients who have been diagnosed with CLD, especially chronic hepatitis B virus (HBV) and hepatitis C virus (HCV), collected from the Center of Internal Medicine and Cardiology in Marjan Teaching Hospital in Hillah city, Babylon Province, Iraq and other samples were healthy controls. **Materials and Methods:** The CP oxidase activity test was processed by PPD buffer oxidation. Ferritin concentration was tested by enzyme-linked immunosorbent assay. **Results:** There were no significant differences in levels of ferritin between HBV and HCV patient groups, but there are slight increases in the mean of CP activity in the HCV patients. A positive significant correlation was observed between CP activity and ferritin. **Conclusion:** 1. CP activity increases in CLD patient and in HCV more than HBV. 2. CP activity is affected by viral load so it increases in positive viral load patients compared with negative viral load patients. 3. There was a positive correlation between CP activity and ferritin. 4. Serum ferritin is not affected by CLD.

Keywords: Ceruloplasmin, ceruloplasmin activity, chronic liver disease, ferritin

INTRODUCTION

Ceruloplasmin (CP) is a copper-containing glycoprotein found in the globulin portion of human blood serum. It has a molecular mass of 132 kDa.^[1] CP, a glycoprotein secreted by hepatocytes, carries more than 90% of the copper in the bloodstream of healthy people. According to studies, CP is an effective antioxidant that prevents lipid peroxidation by removing oxygen.^[2] The unique feature of CP is that it is the only ferroxidase present in a soluble status. This allows CP to perform various fundamental and systemic functions as almost an available protein in the bloodstream. As a consequence of their essential function in exporting and detoxifying iron, CP mRNA transcription, and so production under iron deficiency and oxidative stress, is up-regulated.^[3] CP actually acts by matching up to various Cu sites on multiple substrates. However, it is best known to be effective to convert Fe²⁺ to

Fe³⁺, and CP is the most widely known ferroxidase found in blood plasma.^[4] CP main function is to promote the iron redox transitions necessary for iron transportation by membranes of transferrin/transferrin receptor-mediated. As a multi-copper-oxidase, the CP is capable by type I Cu ions in domains 2, 4, and 6 of accepting electrons and then supplies them to the trinuclear Cu-cluster in which O₂ binds and reduced to produce two H₂O molecules.^[5] CP can suppress reactive oxygen species produced through the ferrous ion mediation, so it is an excellent antioxidant in the body. Meanwhile, CP may also decrease

Address for correspondence: Mr. Mohammed Hussein Habeeb, College of Medicine, University of Babylon, Babylon, Hillah, Iraq. E-mail: thelovemohammed@gmail.com

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metal toxicity through the tissue of the body in order to avoid tissue and function defects by antioxidation.^[6] CP has the association of the Haber–Weiss reaction with hydroxyl radical's production, which then inhibits lipid peroxidation initiation. CP has enzymatic function also known as extracellular superoxide dismutase species (SOD) that neutralizes radicals of superoxide anions.^[7] CP has also been shown that it, like superoxide dismutase (SOD), also suppresses xanthine oxidase. This carries out radical anion removing; SOD can scavenge O_2^- generated from reduction to H_2O_2 and Fenton reaction subsequently submitted to Fe^{+2} ($Fe^{+2} + H_2O_2 \rightarrow Fe^{+3} + OH^- + OH^+$). CP finally binds to Fe^{2+} and oxidizes Fe^{2+} to Fe^{3+} , taking property of ferroxidase activity and eventually eliminating the product of this reaction.^[8] Nitric oxide production is used to battle free radicals which can also stimulate CP by triggering the nitric oxide synthase enzymes expression in the cell that can substantially suppress lipid peroxidation, further reducing the efficient hydrolysis of red blood cells induced by Cu^{+2} ions.^[9] Our goal was to develop a model based on the integration of routine clinical parameters with the activity of CP and ferritin to accurately know the effect of chronic liver disease (CLD) on the activity of CP and also to understand the status of CP in CLD patients, which could yield important information regarding the overall oxidative stress and iron storage and its management as a biomarker to predict fibrosis or cirrhosis.

MATERIALS AND METHODS

Out of a total of 86 samples, 50 samples included patients who have been diagnosed with CLD, especially chronic hepatitis B virus (HBV) and hepatitis C virus (HCV), collected from the Center of Internal Medicine and Cardiology in Marjan Teaching Hospital in Hilla city, Babylon Province, Iraq during October 2020–April 2021. In accordance with the background inspection and clinical and biochemical investigations, patients are already diagnosed with CLD by the physician. A second group of generally healthy people was included as the control group (36). Each patient's history, including their ages, length of disease, and medical history, was collected. The samples were split into two groups: patient and control groups.

The CP oxidase activity test was processed by PPD buffer oxidation under certain conditions such as pH, time, and temperature, similar to the method of Sunderman *et al.*^[10]

Ferritin concentration was tested by an enzyme-linked immunosorbent assay kit from company Bioassay (China).

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol

and subject information and consent form were reviewed and approved by a local Ethics Committee.

RESULTS

In this study, 50 patients with CLD were enrolled, with 33 cases of HCV (66%) and 17 cases of HBV (34%). The results in Table 1 showed that there were no significant differences in the ferritin concentration between HBV and HCV patient groups, but there is a slight increase in the mean of CP activity in HCV patients (46.0688 ± 19.7557), which is considered to be statistically significant (P -value=0.004).

For patients who took antiviral drug, viral load became zero and labeled as negative viral load, whereas patients who have viral load are labeled as positive in both types of hepatitis (B and C).

The parameters including CP activity and ferritin were compared between patients with positive and negative viral loads. Table 2 shows that there were no significant differences in levels of ferritin between patients with positive and negative viral loads, but there is an increase in the mean of CP activity in the positive viral load patients, which is considered to be statistically significant (P -value=0.004).

HBV patients

The parameters including activity and ferritin compared between HBV patients with viral load >300 and <300 in Table 3 show that there were no significant differences in the concentration of ferritin between HBV patients with viral load more and less than 300, but there is a slight decrease in the mean of CP activity in patients with HBV viral load >300 , which is considered to be statistically significant (P -value=0.01).

HCV patients

The parameters including CP activity and ferritin compared between HCV patients with viral load $>10,000$ and <10000 in Table 4 show that there were no significant differences in the concentration of ferritin between HCV patients with viral load more and less than 10,000, but

Table 1: Different levels of CP activity and ferritin between patient groups

Parameter	Study group	No.	Mean $\pm$ SD	P-value
CP activity (mg/dL)	Hepatitis B patients	17	39.6812 ± 14.4601	0.004
	Hepatitis C patients	33	46.0688 ± 19.7557	
Ferritin (ng/dL)	Hepatitis B patients	17	75.6471 ± 22.21751	0.717
	Hepatitis C patients	33	73.4545 ± 19.0412	

Table 2: Different levels of CP activity and ferritin between patient-positive viral load and -negative viral load

Parameter	Study group	No.	Mean $\pm$ SD	P-value
CP activity (mg/dL)	Positive viral load	40	47.3763 $\pm$ 18.2266	0.004
	Negative viral load	10	28.3889 $\pm$ 9.2697	
Ferritin	Positive viral load	40	74.5500 $\pm$ 21.89831	0.754
	Negative viral load	10	72.2222 $\pm$ 3.52767	

Table 3: Different levels of CP activity and ferritin between HBV patients with viral load more and less than 300

Parameter	Study group	No.	Mean $\pm$ SD	P-value
CP activity (mg/dL)	HBV viral load >300	5	36.8800 $\pm$ 3.67655	0.01
	HBV viral load <300	7	51.4971 $\pm$ 14.0489	
Ferritin	HBV viral load >300	5	69.2000 $\pm$ 9.78264	0.319
	HBV viral load <300	7	84.4286 $\pm$ 27.80202	

Table 4: Different levels of CP activity and ferritin between HCV patients with viral load more and less than 10,000

Parameter	Study group	No.	Mean $\pm$ SD	P-value
CP activity (mg/dL)	HCV viral load >10,000	13	57.3624 $\pm$ 21.94939	0.003
	HCV viral load <10,000	15	38.5907 $\pm$ 15.02296	
Ferritin	HCV viral load >10,000	13	78.769 2 $\pm$ 23.59786	0.213
	HCV viral load <10,000	15	66.400 $\pm$ 18.415	

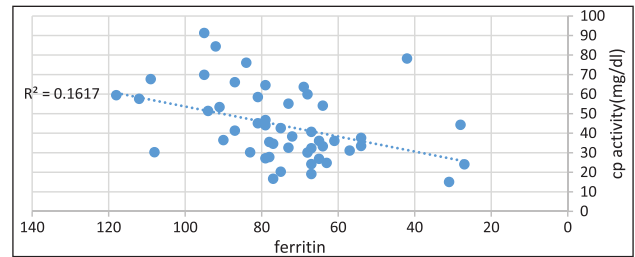
there was an increase in the mean of CP activity in patients with HCV with viral load >10,000, which is considered to be statistically significant (P -value=0.003).

Parameter correlations

A positive significant correlation was observed between CP activity and ferritin at $P \leq 0.01$, which is equal to 0.002 at $r^2=0.16$, as show in Figure 1.

DISCUSSION

CP is synthesized in the liver in *apo* (devoid of copper) and *holo* (replete with copper) forms in more or less equal quantities.^[11] The *holo* form, which is a functional CP enzyme, has a half-life of ~ 5 days,^[12] whereas that of the *apo* form is estimated to be <6 h.^[13] Ebara *et al.*^[14] showed that copper level in liver parenchyma was higher in HCV-positive patients with chronic hepatitis or cirrhosis, which leads to increased oxidation activity function of CP. It is hypothesized that increases in liver copper result in enhanced metalation of the CP protein, leading to a

**Figure 1: Correlation between ferritin and CP activity**

higher proportion of the *holo* form of the enzyme,^[15] and this hepatic copper overload may contribute to the development of hepatocellular carcinoma.^[14]

CP was a new marker, negatively correlated with liver fibrosis and recommended to consider as a biomarker for liver fibrosis. The mechanism whereby CP decreases during fibrosis increase could be due to a selective decrease of hepatocyte synthesis.^[16] It was found in the previous study that there were no relations between CP concentration and CLD that is caused by HBV and HCV; in addition, a novel increase in CP activity of HCV patients was found when compared with HBV patients.^[17] Also, the advancement of imaging techniques such as transient elastography, acoustic power impulsion,^[18] ultrasound (US), computed tomography,^[19] and magnetic resonance imaging (MRI) has in some ways been supplemented with liver biopsy (MRI).^[20] However, high costs of (or limited access to) advanced imaging techniques in the evaluation of liver fibrosis in developing countries can be an additional limitation, which may prevent their use. In addition, a novel technique for predicting liver fibrosis in CHB patients with normal or minimally increased ALT was established on the basis of routine serum markers (especially GGT plus CP levels).^[16] To the best of our knowledge, this is the first study to explore the importance of CP oxidase activity in CLD patients in Babylon, including CHB and CHC at various stages of treatment.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Orzheshkovskiy VV, Trishchynska MA. Ceruloplasmin: Its role in the physiological and pathological processes. *Neurophysiology* 2019;51:141-9. doi: 10.1007/s11062-019-09805-9
- Kang N-L, Zhang J-M, Lin M-X, Chen X-D, Huang Z-X, Zhu Y-Y, *et al.* Serum ceruloplasmin can predict liver fibrosis in hepatitis B virus infected patients. *World J Gastroenterol* 2020;26:3952-62. doi: 10.3748/wjg.v26.i27.3952.
- Tapryal N, Mukhopadhyay C, Das D, Fox PL, Mukhopadhyay CK. Reactive oxygen species regulate ceruloplasmin by a novel mRNA decay mechanism involving its 3'-untranslated region implications in neurodegenerative diseases. *J Biol Chem* 2009;284:1873-83.

4. Vashchenko G, MacGillivray RT. Multi-copper oxidases and human iron metabolism. *Nutrients* 2013;5:2289-313.
5. Haberska K, Vaz-Dominguez C, De Lacey AL, Dagys M, Reimann CT, Shleev S. Direct electron transfer reactions between human ceruloplasmin and electrodes. *Bioelectrochemistry* 2009;76:34-41.
6. Lazzaro M, Bettegazzi B, Barbariga M, Codazzi F, Zacchetti D, Alessio M. Ceruloplasmin potentiates nitric oxide synthase activity and cytokine secretion in activated microglia. *J Neuroinflamm* 2014;11:164.
7. Shanin YN, Shanin VY, Zinov'ev EV. Antioxidant therapy in clinical practice [in Russian]. St. Petersburg: 2003.
8. Ganini D, Canistro D, Jang J, Stadler K, Mason RP, Kadiiska MB. Ceruloplasmin (ferroxidase) oxidizes hydroxylamine probes: Deceptive implications for free radical detection. *Free Radic Biol Med* 2012;53:1514-21.
9. Wang B, Wang X-P. Does ceruloplasmin defend against neurodegenerative diseases? *Curr Neuroparmacol* 2019;17:539-49.
10. Sunderman Jr FW, Nomoto S. Measurement of human serum ceruloplasmin by its p-phenylenediamine oxidase activity. *Clin Chem* 1970;16:903-10.
11. Hellman NE, Gitlin JD. Ceruloplasmin metabolism and function. *Annu Rev Nutr* 2002;22:439-58. doi: 10.1146/annurev.nutr.22.012502.114457.
12. Mazumder B, Sampath P, Fox PL. Translational control of ceruloplasmin gene expression: Beyond the IRE. *Biol Res* 2006;39:59-66. doi: 10.4067/S0716-97602006000100007.
13. Matsuda I, Pearson T, Holtzman NA. Determination of apoceruloplasmin by radioimmunoassay in nutritional copper deficiency, Menkes' kinky hair syndrome, Wilson's disease, and umbilical cord blood. *Pediatr Res* 1974;8:821-4.
14. Ebara M, Fukuda H, Hatano R, Yoshikawa M, Sugiura N, Saisho H, *et al.* Metal contents in the liver of patients with chronic liver disease caused by hepatitis C virus. Reference to hepatocellular carcinoma. *Oncology* 2003;65:323-30.
15. Ranganathan PN, Lu Y, Jiang L, Kim C, Collins JF. Serum ceruloplasmin protein expression and activity increases in iron-deficient rats and is further enhanced by higher dietary copper intake. *Blood* 2011;118:3146-53.
16. Zeng D-W, Dong J, Jiang J-J, Zhu Y-Y, Liu Y-R. Ceruloplasmin, a reliable marker of fibrosis in chronic hepatitis B virus patients with normal or minimally raised alanine aminotransferase. *World J Gastroenterol* 2016;22:9586.
17. Habeeb MH, Ewadh MJ, Mousa MJ. Ceruloplasmin concentration in patients with chronic liver disease. *Ann Rom Soc Cell Biol* 2021;25:2332-42.
18. Li S-M, Li G-X, Fu D-M, Wang Y, Dang L-Q. Liver fibrosis evaluation by ARFI and APRI in chronic hepatitis C. *World J Gastroenterol* 2014;20:9528.
19. Castera L, Chan HL, Arrese M, Afdhal N, Bedossa P, Friedrich-Rust M, *et al.*; European Association for Study of Liver; Asociacion Latinoamericana para el Estudio de l Hígado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. *J Hepatol* 2015;63:237-64.
20. Park HS, Kim YJ, Yu MH, Choe WH, Il Jung S, Jeon HJ. Three-tesla magnetic resonance elastography for hepatic fibrosis: Comparison with diffusion-weighted imaging and gadoxetic acid-enhanced magnetic resonance imaging. *World J Gastroenterol* 2014;20:17558.