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CLINICAL STUDY

Plasma copper and ceruloplasmin in patients with alcoholic liver steatosis

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Abstract: Background: In blood plasma, copper is transported in form of ceruloplasmin. Ceruloplasmin is not only a simple transport protein, it is a multifunctional protein with other various physiological functions. At least a part of these functions is connected with ceruloplasmin's enzymatic activity.
Aim of the study: The effect of alcoholic fatty liver on the metabolism of copper and ceruloplasmin was studied.
Material and methods: Patients suffering from alcoholic liver steatosis were enrolled in the study. The serum levels of copper, apoceruloplasmin and oxidase activity of ceruloplasmin were determined.
Results: The copper level in patients with liver steatosis was moderately decreased in comparison to healthy controls. The difference in apoceruloplasmin levels between patients with liver steatosis and healthy controls was not significant, but the specific activity of ceruloplasmin in patients was significantly decreased in comparison to controls (0.59 vs 0.82, $p<0.001$). The decreased specific activity of ceruloplasmin in spite of normal levels of apoceruloplasmin in patients with alcoholic liver steatosis suggested some problems in copper metabolism in these patients.
Conclusions: The results of our study showed disturbances in copper and ceruloplasmin metabolism in patients with alcoholic liver steatosis. We can conclude that the determination of blood plasma ceruloplasmin level on the basis of its enzymatic activity is better than a simple determination of apoceruloplasmin *(Tab. 2, Ref. 14).* Full Text (Free, PDF) *www.bmj.sk.*
Key words: copper, ceruloplasmin, ceruloplasmin oxidase activity, fatty liver, alcohol liver disease.

Copper is an essential trace element, which is an important catalyst for heme synthesis and iron absorption. Copper plays an essential role in mitochondrial function, detoxification of free radicals, neurotransmitter synthesis, cross-linking of connective tissue, and cellular iron metabolism (Wessling-Resnick, 2002). The major fraction of copper being present in blood plasma is integrated in the molecule of ceruloplasmin. Ceruloplasmin belongs to the family of multicopper oxidases. These enzymes are characterized structurally by the presence of three different copper sites classified on the basis of their spectroscopic features (Malkin and Malmstrom, 1970). Over 95 % of plasma copper is bound to ceruloplasmin. Although its precise physiological roles are still unknown, ceruloplasmin's role in iron homeostasis is generally accepted. Frieden demonstrated that human serum ceruloplasmin had a considerable ferroxidase activity and that this protein was able to mobilize iron from liver with subsequent oxidation of ferrous iron and incorporation of the ferric product into apotransferrin (Osaki et al, 1971). A further activity of ceruloplasmin that is worth mentioning is at the border between regulatory and enzymatic functions. Due to its ability to react with, and scavange oxygen species such as superoxide and hydrogen peroxide, ceruloplasmin always has been considered as a type of plasma antioxidant (Halliwell and Gutteridge, 1990).

Chronic alcohol consumption is the major cause of liver disease in the Western world. Consumption of alcohol above a certain daily threshold results in alcoholic fatty liver in almost 100 % of cases. Many observations have led to "multi-hit" hypothesis in the pathophysiologic sequelae of alcoholic liver disease, wherein the "first-hit" involves lipid accumulation in hepatocytes (steatosis), followed by "second-hit" that lead to more serious conditions such as alcoholic steatohepatitis, cirrhosis and cancer. It should be pointed out that the prerequisite for the development of alcoholic steatohepatitis is alcoholic fatty liver (steatosis). It is probable that oxidative stress plays an important role in the development of alcoholic liver diseases. Metabolism of alcohol by CYP2E1 in conjunction with free iron generates reactive free radicals that can lead to oxidative stress, and like acetaldehyde, to the formation of adducts. Oxidative stress leads to peroxidation of phospholipid constituents of plasma and intracellular membranes resulting in necrosis and/or apoptosis.

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Tab. 1. Results of routine hepatological laboratory tests.

Parameter	ALT μkat.l^{-1}	AST μkat.l^{-1}	GMT μkat.l^{-1}	Bilirubin μmol.l^{-1}	Albumin g.l^{-1}	CHE μkat.l^{-1}
Liver steatosis	0.83±0.06	0.67±0.09	3.15±0.38	17.8±1.2	42.8±2.5	81.3±12.1
Control group	0.52±0.04	0.48±0.05	0.55±0.09	12.8±1.5	45.2±3.2	65.8±8.9
Statistical significance	$p<0.05$	NS	$p<0.01$	$p<0.05$	NS	$p<0.05$

Results are given as mean±SEM, ALT – alanine aminotransferase, AST – aspartate aminotransferase, GMT – gamma-glutamyltransferase, CHE – cholinesterase, NS – not significant

Tab. 2. Copper and ceruloplasmin plasma levels in controls and patients with alcoholic fatty liver.

Parameter	Copper μmol.l^{-1}	Cpl-apo mg.l^{-1}	Cpl-E activity mg.l^{-1}	Cpl-E/Cpl-apo	Cu/Cpl-apo μmol.l^{-1}/mg
Controls	17.58±1.31	341.4±6.7	283.3±10.8	0.830±0.024	0.052±0.002
Liver steatosis	13.83±1.05	375.4±28.5	224.3±31.1	0.597±0.053	0.037±0.003
Statistical significance	$p<0.002$	NS	$p<0.05$	$p<0.001$	$p<0.002$

Results are given as mean±SEM. Cpl – ceruloplasmin, Cpl-apo – ceruloplasmin apoprotein, Cpl-E – ceruloplasmin as enzyme, Cu – copper

The purpose of this study was to examine the effect of alcoholic fatty liver on serum ceruloplasmin and copper levels.

Methods

The patients group consisted of 48 patients (32 men+16 women), mean age 46.5 years (range 31–67 years) with histologically verified liver steatosis. The control group consisted of 42 healthy persons (students and blood donors), 30 men and 12 women, mean age 38.8 years, who showed no abnormalities on the basis of ordinary physical and laboratory tests.

Blood serum levels of routine biochemical parameters (activities of alanine aminotransferase – ALT, aspartate aminotransferase – AST, gamma-glutamyl transferase – GMT, cholinesterase – CHE and serum levels of albumin and bilirubin) and serum levels of copper and ceruloplasmin were analyzed. Ceruloplasmin was determined immunochemically as a protein by means of electroimmunodiffusion of monospecific antibodies (SwAHu-Clp/IDP, USOL, Praha) according to Laurell (1966). The enzymatic activity of ceruloplasmin was estimated as polyphenoloxidase at 37 °C using p-phenylene-diamine hydrochloride as a substrate (Přibyl, 1978). The total serum copper was determined by atomic absorption spectrometry (Varian AA-475, Varian Techtron Pty. Ltd., Australia) with flameless atomization (Carbon rod atomizer CRA-90, Varian Pulse Engineering, Ltd., Ireland).

While the data did not have the Gaussian distribution, nonparametric tests were used (Wilcoxon's test for comparing populations).

Results

Routine biochemical parameters of activities of aminotransferases were slightly, but significantly increased in comparison with the control group. The activities of cholinesterase and gamma-glutamyl transferase were also significantly increased ($p<0.001$). The serum level of albumin was in its physiological range and there was no change in comparison with the control group. Bilirubin was also in its physiological range (Tab. 1).

The assessment of copper status in patients with fatty liver was done by means of parameters as follows: the total serum level of copper, the concentration of immunoreactive apoceruloplasmin, and the oxidase activity of ceruloplasmin. From the enzymatic activity of ceruloplasmin and the concentration of ceruloplasmin apoprotein we calculated the specific activity of ceruloplasmin (activity per unit mass of ceruloplasmin protein). Table 2 shows the results obtained both in the studied group of patients with liver steatosis and the control group. The levels of serum copper in patients with fatty liver were significantly lower in comparison with the control group (13.83 μmol.l^{-1} vs 17.58 μmol.l^{-1}, $p<0.002$). The concentration of apoceruloplasmin in patients with fatty liver did not differ from the control group (375.4 mg.l^{-1} vs 341.4 mg.l^{-1}). The level of ceruloplasmin determined according to its oxidase activity was significantly decreased (224.3 mg.l^{-1} vs 281.3 mg.l^{-1}). The specific activity of ceruloplasmin (enzymatic activity/apoceruloplasmin) was also decreased. We analyzed also copper saturation of ceruloplasmin (copper/apoceruloplasmin). The results showed a decreased content of copper in ceruloplasmin in patients with fatty liver when compared to the control group (Tab. 2).

Discussion

Fat accumulation in liver cells is the earliest and most common response to alcohol abuse. In massive steatosis the hepatocytes are uniformly filled by large fat droplets, the cell nucleus may be eccentrically placed and when the cell membranes be-

tween adjacent hepatocytes rupture, fatty cysts are formed. Ultrastructural changes reveal enlarged and distorted mitochondria with shortened cristae containing crystalline inclusions. The endoplasmic reticulum shows vacuolar dilatation and proliferation (Lieber, 1996). The presence of lipid particles in the cytoplasm of hepatocytes alters the ultrastructure of cellular membranes. A damaged liver cell is unable to synthesize phospholipid adequately. The reparative processes are limited especially in cases where the hepatocyte is exposed to a long-lasting stress (Kuntz, 1991). Hepatic steatosis is very often associated with slight alteration of either aminotransferases or gamma-glutamyl transferase, or both (Bellentani and Tiribelli, 2001). The results of our study confirmed changes in activities of aminotransferases and gamma-glutamyl transferase described in sera of patients with fatty liver. Elevated levels of gamma-glutamyl transferase could be also explained by the fact, that our group of patients consisted of patients with alcoholic fatty liver while alcohol is a factor well known to increase blood serum activity of gamma-glutamyl transferase.

There was no change in albumin concentration in the blood serum of our patients with alcoholic fatty liver. This is not surprising because albumin is the most abundant plasma protein and has many important functions. This importance of albumin results in a relatively very large functional reserve in the synthesis of this protein within hepatocytes. There may be an extensive reduction in mass of functioning liver parenchyma before the deficiencies in the synthesis of albumin can be detected.

The activity of serum cholinesterase was slightly increased in our group of patients with liver steatosis. Increased activities of serum cholinesterase were described also in patients with non-alcoholic liver steatosis (Nomura et al, 1986, Thomas, 2000, Turecky et al, 2005).

The significantly decreased levels of copper and ceruloplasmin enzymatic activities suggest some disorders of copper metabolism in patients with alcoholic fatty liver. Decreased ceruloplasmin oxidase activity in spite of no significant changes in ceruloplasmin apoprotein concentration could result from the synthesis of a functionally defective ceruloplasmin molecule. The oxidase activity of ceruloplasmin is derived from the reduction of selected copper atoms in oxidase sites of the holoprotein. It is possible, that the decreased content of copper in ceruloplasmin molecules could be the cause of decreased ceruloplasmin enzymatic activity in our patients with fatty liver. This hypothesis is supported by the decreased ratio of Cu/Cp found in our group of patients. These results are in agreement with Milne's studies (1994, 1999), the fact of which suggests that in adults the specific oxidase activity of ceruloplasmin is a better indicator of copper status than serum concentrations of copper or ceruloplasmin.

Finding of decreased enzymatic activity in sera of patients with liver steatosis is interesting because ceruloplasmin is one of blood plasma factors with antioxidant activity and it is generally accepted that oxidative stress and reactive oxygen radicals participate in the pathogenesis of chronic alcoholic hepatopathies. Disturbed ceruloplasmin functional activity could participate in increased oxidative stress in these patients as well as in the development of steatohepatosis from fatty liver. However from the diagnostic point of view, our results suggest that the determination of blood plasma ceruloplasmin level on the basis of its enzymatic activity is preferable to the simple determination of apoceruloplasmin.

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