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Evaluation of Ceruloplasmin - A Potential Biomarker in Chronic Heart Failure

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Abstract

Background: Ceruloplasmin (Cp) is an acute phase protein with plasma copper binding properties and is involved in inflammatory processes and oxidative stress. Cp may play a role in the pathophysiology of heart failure.

Study objective: To analyze relation of Cp with disease severity in patients with chronic stable heart failure as assessed by clinical and laboratory parameters.

Patients and methods: Serum levels of Cp, NT-proBNP and other biochemical parameters were analyzed in 164 consecutive patients with chronic stable heart failure (CHF group). Consecutive 64 patients with arterial hypertension (AH group) without heart failure served as a control group.

Results: Mean Cp level in CHF group was 230 mg/l and 200 mg/l in AH group ($p<0.001$), mean NT-proBNP was 1843 pg/ml in CHF and 139 pg/ml AH ($p<0.001$). Cp correlated with NT-proBNP in HF ($r=0.323$, $p<0.001$) in CHF group significantly and in AH group not significantly ($r=0.222$, $p=0.08$). Cp correlated significantly with total bilirubin ($r=0.213$, $p=0.0067$) and with albumin (inverse correlation $r=-0.273$, $p=0.0005$). Cp level correlated with functional class NYHA ($r=0.230$, $p=0.0034$) and LV EF (inverse correlation–0.237, $p=0.0025$) too. Cp correlated in both groups ($n=228$) with NT-proBNP significantly ($r=0.349$, $p<0.0001$).

Conclusion: Cp in patients with stable CHF correlates with disease severity as assessed by the level of NT-proBNP, functional class NYHA, LV EF, bilirubin and albumin.

Keywords: Ceruloplasmin; Chronic heart failure; Disease severity; Arterial hypertension

Introduction

Ceruloplasmin (Cp) is a glycoprotein synthetized mainly by hepatocytes, whose functions include the acute phase inflammation reactant, the serum copper transport and is involved in iron metabolism through its ferroxidase activity [1]. Inflammation and oxidative stress might explain the role of Cp in the pathophysiology of heart failure. Epidemiologic studies showed association of elevated Cp levels with the risk of atherosclerosis, coronary heart disease, myocardial infarction and ischemic heart failure [2-5]. The Atherosclerosis Risk in Communities (ARIC) Study revealed association of elevated Cp levels with the risk of incident heart failure (HF), HF mortality and risk of cardiovascular disease (CVD) in healthy individuals [6]. In the patients with established chronic heart failure (CHF), Cp levels correlated with the severity of heart failure and with B-natriuretic peptide levels, and predicted all-cause mortality [7].

Correlation of Cp levels with natriuretic peptides in heart failure has been already described. Role of Cp in the assessment of disease severity in patient with stable chronic heart failure has not been yet evaluated.

Patients and Methods

Between October 2014 and May 2015, a total of 164 consecutive patients with HF and reduced left ventricle ejection fraction (LVEF) and New York Heat Association (NYHA) functional class II-IV from tertiary care heart failure outpatient clinic were recruited to the study. Patients with stable heart failure with no change of medical therapy within last three months and no history of HF hospital admission at least six months before study entry were included in the study. Control group comprised of 64 consecutive patients with arterial hypertension (AH), followed at a hypertension clinic and were recruited in the same time interval. Patients with evidence of infection, chronic inflammatory disease, active rheumatic disease, advanced chronic kidney disease and liver disease were not included in the study.

Both patients groups were treated according European heart failure, and hypertension guidelines. All patients enrolled in the study were given informed consent, and the hospital ethics committee approved the study protocol.

Blood samples for biomarkers and standard laboratory parameters were collected in the morning in fasting patients. Serum levels Cp, NT-proBNP and other biochemical parameters were analyzed. Ceruloplasmin concentration was measured by turbidimetric immunoassay on AU 400 analyzer (Olympus Life and Material Science

Europa GmbH, Hamburg, Germany). Normal value range in healthy subjects is 220-400 mg/l. NT-proBNP was measured using a validated, commercially available sandwich electro-chemiluminescence immuno-assay on Cobas e411 analyzer (Roche Diagnostics, Mannheim, Germany) using established methodology. Normal value range in healthy subjects is <125 pg/ml. Other biochemical analyses were realized on the analyzer Unicel DxC 800 (Beckman Coulter Company, Germany). Echocardiography studies were realized on the same clinic visit day. An echocardiographic examination of the each patient was performed using a broadband transducer with a transmitting frequency from 1.7 to 4.0 MHz on commercially available equipment (Vivid 7, GE, USA). Left ventricle ejection fraction (LV EF) was measured by Simpson´s method. New York Heart Association functional class was evaluated and recorded in each CHF patient on the day of HF clinic visit.

Continuous variables were given as mean ± standard deviation and median, categorical variables were defined as percentage. Student´s t-test was used for continuous variables and correlation between Ceruloplasmin and other parameters was evaluated by Spearman´s correlation coefficient r. Statistical significance was defined as p value less than 0.05.

Results

	CHF, n=164			AH, n=64		
parameter	**mean**	**SD**	**median**	**Mean**	**SD**	**median**
Age years	66.5	11	67	65.7	9.01	66
Systolic BP mmHg	135	20	135	146.3	19.74	143
HR bpm	72	12	72	69.4	11.08	68
Height cm	173	9	173	171.5	8.95	171
Weight kg	89	16	89	89.5	18.19	87
NYHA class	1.99	0.60	2		-	-
LV EF %	32	8	30	67	5.5	66
parameter	number	proportion		number	proportion	
CAD	89	54%		17	27%	
MI	78	48%		5	8%	
PCI	60	37%		12	19%	
CABG	30	18%		6	9%	
Hypertension	103	63%		64	100%	
Dyslipidaemia	107	65%		56	88%	
Diabetes mellitus	124	76%		34	53%	

Table1: Patients characteristics and medical history; CHF: Chronic heart failure; AH: Arterial hypertension; SD: Standard deviation; BP: Blood pressure; HR: Heart rate; NYHA: New York Heart Association; LV EF: Left ventricle ejection fraction; CAD: Coronary artery disease; MI: History of myocardial infarction; PCI: History of percutaneous coronary intervention; CABG: History of coronary bypass graph.

parameter	**HF n=164**			**AH n=64**			**Units**	**P value**
	mean	SD	median	mean	SD	median		
Na	137	3.18	137	138.4	2.72	138	mmol/l	=0.003
Creat	104.9	35	96	83.3	15.88	82.5	µmol/l	<0.001
Urea	6.96	3.7	6	5.2	1.86	5	mmol/l	<0.001
Bilirubin	16.61	9.78	15	13.1	5.38	12.55	µmol/l	=0.001
ALT	0.53	0.58	0.4	0.5	0.26	0.48	µkat/l	N.S.
AST	0.36	0.22	0.3	0.4	0.20	0.3,25	µkat/l	N.S.
GGT	0.96	1.10	0.58	0.6	0.6	0.405	µkat/l	=0.004
ALP	1.3	1.04	1.11	1.1	0.35	1.08	µkat/l	N.S.
albumin	39.78	3.73	40	40.7	2.34	40.1	g/l	N.S.
UA	389.85	101.79	386.5	328.3	69.35	335	µmol/l	<0.001
TC	4.40	1.23	4.22	4.8	1.17	4.625	mmol/l	=0.041
HDL chol	1.16	0.33	1.09	1.33	0.34	1.26	mmol/l	=0.001
LDL chol	2.71	1.01	2.47	2.9	1.01	2.805	mmol/l	N.S.
TG	1.84	1.20	1.54	1.8	1.71	1.33	mmol/l	N.S.
Hgb	141.3	14.66	142	145.3	12.27	144	g/l	=0.041
NT-proBNP	1843	305.2	1074	139	14.03	13	pg/ml	<0.001
ceruloplasmin	230	50	230	200	40	210	mg/l	<0.001

Table 2: Biochemical results in CHF and AH groups; Na: Sodium; Creat: Creatinine level; ALT: Alanine Aminotranspherase; AST: Aspartame Aminotranspherase; GGT: Gamaglutamyl transpherase; ALP: Alkalis Phosphatase; UA: Uric Acid; TC: Total Cholesterol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; TG: Triglycerides; Hgb: Hemoglobin; SD: Standard Deviation.

Mean patients´ age in CHF group was 66 yrs, mean LV EF was 32%; ischemic aetiology of HF had 54% patients. Of 164 heart failure patient, 113 (69%) had automatic implantable defibrillator, 35 (21%) had cardiac resynchronization therapy and 67 (41%) had chronic forms of atrial fibrillation. Mean age of control group was 65 yrs, mean LV EF was 67% and patients with AH didn´t have signs or symptoms of heart failure. Patient's characteristics and medical history is shown in the table (Table 1). Differences of standard laboratory results between heart failure group and hypertension group are shown in the table

(Table 2). In the heart failure patients, creatinine level, urea, total bilirubin, gamaglutamyl transpherase and uric acid levels were significantly higher compared to hypertension group. Mean sodium level, total cholesterol, LDL cholesterol and triglyceride and hemoglobin levels were significantly lower in heart failure group compared to hypertension group. NT-proBNP and Cp levels were significantly higher in heart failure patients compared to hypertension group.

Cp correlated significantly with NT-proBNP in heart failure group, but not in hypertension group (Figure 1). When analyzing both patients groups together, ceruloplasmin correlated significantly with NT-proBNP too (Figure 2).

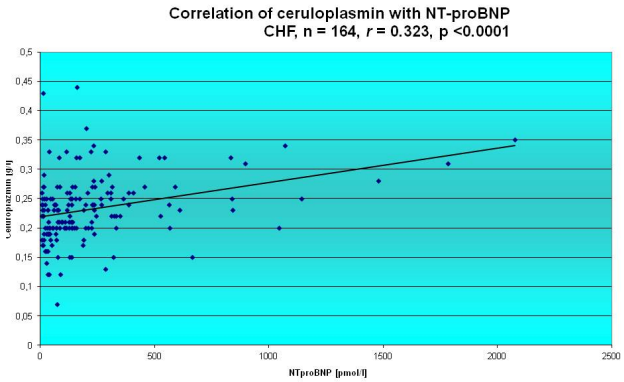

Figure 1: Ceruloplasmin correlated significantly with NT-proBNP in heart failure group.

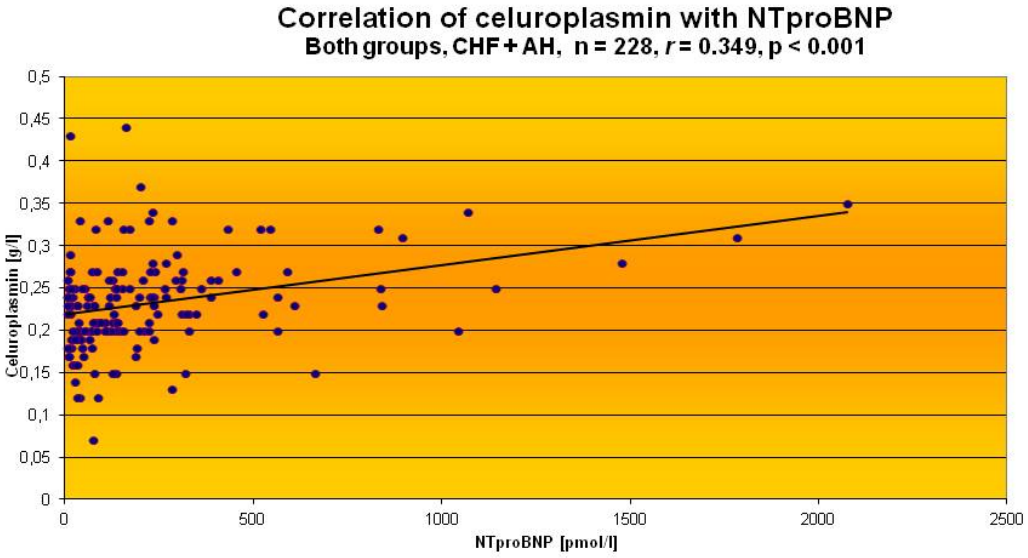

Figure 2: Ceruloplasmin correlated significantly with NT-proBNP.

NT-proBNP correlated with other laboratory parameters, including: parameters of kidney function: urea and creatinine, and correlated significantly with bilirubin and uric acid level.

Significant inverse correlation of NT-proBNP with albumin, total cholesterol and hemoglobin was revealed too. NT-proBNP correlated with age, NYHA class and inversely with LV EF. Cp correlated

significantly with total bilirubin level and albumin level (inverse correlation). Ceruloplasmin level correlated with NYHA and with LV EF (inverse correlation), but not with age (Table 3).

Parameters	Correlation coefficient r	Level of significance p	Parameters	Correlation coeficient r	Level of significance p
NTproBNP/urea	0.405	<0.0001	ceruloplasmin/ urea	0.136	=0.0840
NTproBNP/creatinine	0.311	<0.0001	ceruloplasmin/ creatinin	0.024	=0.7643
NTproBNP/bilirubin	0.304	<0.0001	ceruloplasmin/ bilirubin	0.213	=0.0067
NTproBNP/albumin	-0.336	<0,0001	ceruloplasmin/ albumin	-0.273	=0.0005
NTproBNP/UA	0.266	<0,001	ceruloplasmin/ UA	0.097	=0.2208
NTproBNP/chol	-0.263	<0.001	ceruloplasmin/ chol	0.000	=0.9964
NTproBNP/Hgb	-0.168	0.0334	ceruloplasmin/ Hgb	0.066	=0.4009
NT-proBNP/ NYHA	0.347	<0.001	ceruloplasmin/ NYHA	0.230	=0.0034
NT-proBNP/ LV EF	-0.359	<0.001	ceruloplasmin/ LV EF	-0.237	=0.0025
NT-proBNP/ age	0.323	<0.001	ceruloplasmin/ age	-0.041	=0.2459

Table 3: Correlation of NT-proBNP and cerulosplasmin with other laboratory parameters and LVEF, NYHA class and age; UA: Uric Acid; Hgb: Hemoglobin; NYHA: New York Heart Association; LV EF: Left Ventricle Ejection Fraction; Chol: Total Cholesterol.

Discussion

Our study confirms the possible role of ceruloplasmin in the pathophysiology of heart failure. The role of ceruloplasmin in the development of heart failure might be an attractive subject of research. Epidemiological studies showed association of elevated Cp levels with risk of ischemic heart failure and the risk of incident heart failure and mortality for HF in healthy subjects [8]. The main evidence is based on the results of The Atherosclerosis Risk in Communities (ARIC) Study [6]. The ARIC study was a prospective population study of cardiovascular disease in more than 15,000 middle-aged adults from four U.S. communities in 1987-1989. After exclusion of the patients with prevalent heart failure, analysis comprised 9,240 subjects with baseline ceruloplasmin available. The study investigated association of Cp with incident heart failure, coronary heart disease and all-cause mortality. The mean follow-up was 10,5 yrs. During follow-up, a total of 752 subjects were hospitalized for HF, 1275 patients died and 1234 had cardiovascular events. After adjusting for traditional risk factors, C-reactive protein and NT-proBNP, higher Cp levels were associated with incident heart failure (hazard ratio HR 1,44, 95% confidence interval 1,13-1,83) and mortality (HR 1,38, CI 1,11-1,63).

We investigated the association of ceruloplasmin, an acute phase protein with the severity of disease in patients with established heart failure and stable course of the disease. We confirmed significant correlation of ceruloplasmin with standard HF biomarker: NT-proBNP. This association has been previously described previously [7,9,10]. In a study of Hammadah Cp levels were measured in 890 patients with stable heart failure undergoing coronary angiography [7]. The diagnosis of heart failure was based on medical history (direct asking) or by reviewing medical records. Patients with recent acute coronary syndrome were excluded from the study. The mean Cp level at baseline was 26.6 mg/dl, this value is higher than in our HF group (mean Cp level 230 mg/l). The study of Hammadah showed weak correlation of Cp concentration with B-type natriuretic peptide (C-terminal fragment of proBNP) with correlation coeficient r=0.187, p<0.001). In our study we used N-terminal fragment of proBNP (NT-proBNP), which is considered to be a more important type form of natriuretic peptide for the assessment of subjects with stable heart failure [11]. The main results of a study of Hammadah were that higher Cp levels were associated with increased five years all-cause mortality (hazard ratio 1.9, 95% CI1.4-2.8, p<0.001). After adjusting to traditional risk factor for coronary artery disease, body mass index, BNP level, left ventricle ejection fraction, heart rate and other, higher

Cp level remained independent risk factor of increased mortality (HR1.7, 95% CI 1,1-2,6, p<0,05).

Unfortunately, Cp levels were not studied in relation to BNP or NT-proBNP in an important study of Xu and co-authors [12]. The aim of that study was to evaluate the role of Cp with the extent of heart failure in patients with ischemic and non-ischemic cardiomyopathy. The diagnosis of heart failure was based on admission medical records and the extent of heart failure was defined according to NYHA classification. A total number of 202 patients underwent coronary angiography and was divided to a group of 78 patients with coronary artery disease (CAD) (ischemic HF group) and to 124 patients group without CAD (non-ischemic cardiomyopathy). Total number of 94 subjects without heart disease was included in a control group. Cp levels were higher in both HF groups compared to controls, and were related to functional NYHA class only in non-ischemic HF. Cp correlated with C-reactive protein and inversely correlated with LVEF. Mean Cp concentration in control group was 250 mg/l, in ischemic HF was 313 mg/l and in non-ischemic HF 328 mg/l. These values are somewhat higher compared to results of our study.

There are several key findings from our present study of the subjects with stable chronic heart failure. The results showed significant correlation of ceruloplasmin with total bilirubin and inverse correlation of ceruloplamin with albumin. Both bilirubin and albumin level are considered to be prognostic indicators in HF. Bilirubin levels are typically increased in acute heart failure [13]. Bilirubin and albumin correlated with NT-proBNP and predicted outcome in advanced heart failure [14,15]. The association of ceruloplasmin with bilirubin and albumin may not be explained by production of these molecules by hepatic cells. The liver is major source of serum ceruloplasmin and the synthesis of ceruloplasmin in hepatic cells is increased by pro-inflammatory factors, e.g.: interleukin-6 and tumor necrosis factor-α [16].

Another finding of our study is association of ceruloplasmin with NT-proBNP in patients with arterial hypertension. This correlation was not statistically significant. When analyzed in two groups of patients together, correlation of ceruloplasmin with NT-proBNP, in subjects with stable heart failure and arterial hypertension (n=228) was highly significant. This is an interesting finding which may reflect close relation of hypertension and heart failure. Arterial hypertension is one of the major independent risk factors of development of heart failure.

There is only small evidence of the role of ceruloplasmin in arterial hypertension. In a study of Vasconcelos and co-authors. The authors of that study investigated redox imbalance in arterial hypertension using superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), vitamin C, transferrin, ceruloplasmin, malondialdehyde (MDA) [17]. Cp levels together with GPx were significantly higher in a group of patients with arterial hypertension compared to healthy controls (p=0.015 and p=0.001 respectively).

Mean Cp level was 38.6 mg/dl (386 mg/l), this value is much more higher than observed in our hypertension group.

In a second study of Ke˛dziora-Kornatowska and co-authors investigated the oxidase activity of ceruloplasmin, the production of nitric oxide, and the process of lipid peroxidation in the blood of elderly patients with primary hypertension [18]. The oxidase activity of ceruloplasmin in blood was assessed by colorimetric enzymatic assay. The oxidase activity of ceruloplasmin was significantly higher in elderly hypertensive patients compared to elderly normotensive subjects and significantly decreased after seven days of treatment with angiotensin-converting enzyme inhibitor perindopril in patients with arterial hypertension.

Conclusion

To conclude, our study supports role of ceruloplasmin in chronic heart failure. Ceruloplasmin concentrations are significantly higher in stable heart failure patients than in hypertensive patients and correlate with the severity of cardiac failure as assessed by functional class NYHA, LVEF, NT-proBNP and biochemical parameters of organ damage.

Our study is limited by the small number of subjects. Further studies should confirm our data in a larger number of patient populations addressing the prognostic role of ceruloplasmin as assessed together with NT-proBNP and other biomarkers of heart failure. The evaluation of the role of ceruloplasmin in the arterial hypertension needs further research too.

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