Serum Caeruloplasmin as a Coronary Risk Factor in Patients with Acute Myocardial Infarction with Normal Lipid Profile

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There have been studies demonstrating that serum caeruloplasmin acts as an antioxidant in cardiovascular disease. However, several studies have demonstrated that it acts as an independent risk factor in patients with cardiovascular disease. To ascertain the role of caeruloplasmin in normolipidemic acute myocardial infarction patients, we investigated the correlation between the serum caeruloplasmin level and incidence of cardiovascular disease in individuals with normal lipid profiles. The levels of caeruloplasmin were significantly higher in patient sera than in those of controls ($p < 0.001$) and the difference in the lipid profiles was also significant ($p < 0.001$). These results suggest that caeruloplasmin may act as a prooxidant and appears to be a risk factor in this disease.

Key words —— acute myocardial infarction, normal lipid profile, caeruloplasmin

INTRODUCTION

Coronary artery disease is a major cause of mortality and morbidity in the industrialized world.[1)] Elevated serum caeruloplasmin levels have been found in patients with cardiovascular disorders including arteriosclerosis, abdominal aneurysms, unstable angina, and vasculitis and peripheral artery disease.[2)] Several prospective studies have indicated that the serum copper or caeruloplasmin level may be an independent risk factor for cardiovascular disease.[3–5)] The increased risk has been attributed to the prooxidant function of caeruloplasmin, and recent experimental studies demonstrating the ability of caeruloplasmin to oxidatively modify low-density lipoprotein (LDL)[6)] seem to underline this concept. However, the question has been raised whether elevated caeruloplasmin is not merely an indicator of inflammation, given its acute-phase protein property. Studies have demonstrated the antioxidant property of caeruloplasmin through its oxidase activity, which is directed toward ferrous ions (ferroxidase activity).[7)] Studies have also demonstrated the inhibition of lipid peroxidation by ferrous ion which is also known to be involved in the decomposition of lipid peroxides.[8)] Due to mixed results of the studies conducted earlier, the present study was undertaken. Moreover, earlier studies investigated the association between caeruloplasmin or copper and cardiovascular disorders in patients with hyperlipidemia, but in the present study, the serum caeruloplasmin levels in normolipidemic acute myocardial infarction (AMI) patients were studied due to the dearth of reports related to caeruloplasmin concentration in normolipidaemic individuals.

MATERIALS AND METHODS

Setting Design and Patients —— The study was conducted among 165 patients (123 men and 42 women) with AMI admitted to the Intensive Cardiac Care Unit, Hindustan Institute of Medical Sciences, India. The diagnosis of AMI was established according to the following diagnostic criteria: chest pain lasting for up to 3 hr; electrocardiogram changes (ST elevation of 2 mm or more in at least two leads); and elevation of serum creatine phosphokinase (CPK-MB) and aspartate aminotransferase levels. The control group consisted of 165 age sex and matched healthy volunteers (123 men and 42 women). The study was conducted from April 2002 to August 2006. Informed consent was obtained from all subjects before enrollment in the study, and the protocol was approved, by the Ethical committee of the institute.

Inclusion Criteria —— Patients with a diagnosis of AMI with normal lipid profiles were enrolled.

Exclusion Criteria —— Patients with diabetes mellitus, renal insufficiency, or hepatic disease, or

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who were taking lipid lowering drugs or antioxidant vitamin supplement and current or past smokers were excluded from the study.

Criteria for Normolipidemics —— A normal lipid profile was defined as LDL < 160 mg/dl, high-density lipoprotein (HDL) ≥ 35 mg/dl, total cholesterol (TC) < 200 mg/dl and triglycerides (TG) < 150 mg/dl.[9)]

For biochemical assays 5 ml of blood was collected after an overnight fast. The serum was separated and used for the determination of lipid profiles and caeruloplasmin levels.

Lipid Profile —— TC, TG, and HDL were analyzed enzymatically using kits obtained from Randox Laboratories Limited (Crumlin, UK). Plasma LDL was determined from the values of TC and HDL using the following formula:

$$\text{LDL} = \text{TC} - \frac{\text{TG}}{5} - \text{HDL (mg/dl)}$$

Serum Caeruloplasmin Assay —— The caeruloplasmin assay was performed using the *p*-phenylene diamine method.[10)] The serum caeruloplasmin assay was performed using the *p*-phenylenediamine method. The principle of the assay is based on the oxidation of *p*-phenylenediamine to produce a purple-colored complex with an absorption peak at 530 nm. All chemicals of analytical grade were obtained from Sigma Chemicals (New Delhi, India).

RESULTS AND DISCUSSION

Demographic data on controls and AMI patients are shown in Tables 1 and 2. The differences in age, height, and body mass index (BMI) in control and AMI patient were not significant. The weight and waist circumference were greater in AMI patients than those in the control group ($p < 0.001$, Tables 1 and 2). Systolic and diastolic blood pressure was significantly higher in AMI patients than in controls ($p < 0.05$, Tables 1 and 2).

The lipid profiles and serum caeruloplasmin concentrations are shown in Tables 3 and 4. The TC and TC:HDL ratio, TG, LDL and LDL:HDL ratio, and serum caeruloplasmin were significantly higher ($p < 0.001$) in AMI patients compared with controls (Table 3). A significant difference ($p < 0.001$) was also observed in HDL levels between AMI patients and controls. TC, TC:HDL ratio, TG, and serum caeruloplasmin were significantly higher ($p < 0.001$) in both genders of AMI patients compared with controls (Table 4). A significant difference ($p < 0.001$) in HDL levels between AMI patients and controls was seen only among women (Table 4). LDL and LDL:HDL ratio were significantly higher ($p < 0.001$) in AMI male patients

Table 1. Baseline Variables in Controls and AMI Patients (mean ± S.D.)

Variable	Control (n = 165)	Patients (n = 165)
Age	60.6 ± 4.0	61.8 ± 3.8[†]
Height (cm)	1.6 ± 0.1	1.6 ± 0.1[††]
Weight (kg)	68.3 ± 4.0	72.0 ± 5.4[§]
BMI (kg/m^2)	25.4 ± 1.2	26.2 ± 1.5[‖]
Waist circumference (cm)	93.7 ± 3.6	100.8 ± 6.1[§]
Hip circumference (cm)	100.0 ± 3.2	105.7 ± 5.2[§]
Waist:Hip[*]	0.9	1.0[¶]
Systolic blood pressure (mm Hg)	113 ± 8	136 ± 2[**]
Diastolic blood pressure (mm Hg)	85 ± 7	95 ± 10[**]

[*]Ratio; [†]($p = 0.0037$); [††]($p = 0.2919$); [§]($p < 0.001$); [‖]($p < 0.01$); [¶]($p < 0.02$); [**]($p < 0.05$).

Table 2. Baseline Variables in Men and Women in the Control and AMI Patient Groups (mean ± S.D.)

Variable	Control		Patients	
	Men (n = 123)	Women (n = 42)	Men (n = 123)	Women (n = 42)
Age	60.7 ± 4.1	60.5 ± 2.9	61.5 ± 3.3[†]	62.7 ± 4.97[**]
Height (cm)	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1[††]	1.6 ± 0.1[†††]
Weight (kg)	68.6 ± 3.8	67.5 ± 4.4	71.7 ± 5.3[§]	72.5 ± 5.7[§]
BMI (kg/m^2)	25.3 ± 1.1	25.6 ± 1.5	26.0 ± 1.3[‖]	26.6 ± 1.7[‡‡]
Waist circumference (cm)	93.4 ± 3.4	94.6 ± 4.2	100.2 ± 5.7[§]	102.5 ± 6.8[§]
Hip circumference (cm)	99.7 ± 2.9	100.8 ± 3.8	105.3 ± 5.0[¶]	107.0 ± 5.8[§]
Waist:Hip[*]	0.9 ± 0.0	0.9 ± 0.0	1.0 ± 0.0[¶]	1.0 ± 0.0[§]
Systolic blood pressure (mm Hg)	114 ± 7	118 ± 8	136 ± 13[¶]	132 ± 17[¶]
Diastolic blood pressure (mm Hg)	86 ± 8	87 ± 9	95 ± 15[¶]	94 ± 13[¶]

[*]Ratio; [†]$p = 0.0366$; [††]$p = 0.0081$; [§]$p < 0.001$; [‖]$p = 1.5085$; [¶]$p < 0.05$; [**]$p = 0.0356$; [†††]$p = 0.0170$; [‡‡]$p = 0.0001$.

compared with controls (Table 4).

In the present study, we observed a significant association between high baseline levels of serum caeruloplasmin and the subsequent risk of myocardial infarction. Comparable findings of an elevated risk of myocardial infarction[5] and incidence of coronary heart disease (CHD)[11] among individuals with high levels of serum caeruloplasmin have been reported. Several other studies reported associations of high levels of serum Cu with an elevated risk of increased carotid intima-media thickness,[12] myocardial infarction,[13] mortality from CHD or cardiovascular disease.[3,4] The present studies along with the findings of previous ones indicate that caeruloplasmin could be a prooxidant in normolipidemic AMI patients.

The prooxidant property of caeruloplasmin involves lipid peroxidation. Studies indicate that caeruloplasmin by itself can oxidize LDL *in vitro* and possibly *in vivo*.[6,14] However, accessory factors derived from vascular cells may be modulatory or requisite during lipoprotein oxidation within the vessel wall.[2] Studies have reported about the cardioprotective nature of caeruloplasmin[15] which could protect the myocardial tissue against the deleterious effects of oxygen free radicals. Studies observing the role of transition metal ion-mediated oxidation of LDL molecules centered on the role of human caeruloplasmin in this oxidative process as it is the principal coppercontaining protein in serum. Biochemical studies showed that caeruloplasmin is a potent catalyst of LDL oxidation *in vitro*. The prooxidant activity of caeruloplasmin requires an intact structure, and a single copper atom at the surface of the protein, near histidine (426), is required for LDL oxidation. Under conditions where an inhibitory protein (such as albumin) is present, LDL oxidation by caeruloplasmin is optimal in the presence of superoxide, which reduces the surface copper atom of caeruloplasmin. Cultured vascular endothelial and smooth muscle cells also oxidize LDL in the presence of caeruloplasmin. Superoxide released by these cells is a critical factor regulating the rate of oxidation. The role of caeruloplasmin in lipoprotein oxidation and atherosclerotic lesion progression *in vivo* has not been directly assessed and is an important area for future studies.[16]

Studies have demonstrated significantly higher levels of caeruloplasmin copper and anti-oxLDL in AMI patients. High concentrations of anti-oxLDL suggest an increase in oxidative stress that would contribute to disease severity. The observed correlation of caeruloplasmin with anti-oxLDL suggests the possible prooxidative activity of caeruloplasmin in patients with cardiovascular disease.[17]

Table 3. Lipid Profile and Serum Caeruloplasmin in the Control and AMI Patient Groups (mean ± S.D.)

Variable	Controls (n = 165)	Patients (n = 165)
Total cholesterol[§]	168.6 ± 12.2	186.4 ± 14.0[†]
HDL-cholesterol[§]	50.5 ± 6.8	41.3 ± 4.6[†]
TC:HDL-C[*]	3.4 ± 0.4	4.6 ± 0.6[†]
Triglycerides[§]	107.8 ± 11.5	129.0 ± 12.2[†]
LDL-cholesterol[§]	83.6 ± 11.9	119.4 ± 14.1[†]
LDL:HDL-C[*]	1.9 ± 0.3	2.9 ± 0.5[†]
TG:HDL-C[*]	2.2 ± 0.4	3.2 ± 0.5[††]
Caeruloplasmin[§]	20.5 ± 2.3	51.5 ± 2.3[†]

[*]Ratio; [†]$p < 0.001$; [††]$p = 1.0008$; [§]mg/dl.

Table 4. Lipid Profile and Serum Caeruloplasmin in the Control Group and Male and Female AMI Patients (mean ± S.D.)

Variable	Control (n = 165)		Patients (n = 165)	
	Men (n = 123)	Women (n = 42)	Men (n = 123)	Women (n = 42)
Total cholesterol[**]	168.1 ± 12.1	170.0 ± 12.4	183.8 ± 13.6[†]	194.0 ± 13.0[†]
HDL-cholesterol[**]	49.9 ± 7.3	52.3 ± 4.6	41.8 ± 4.8[††]	39.8 ± 3.4[†]
TC:HDL-C[*]	3.4 ± 0.3	3.3 ± 0.5	4.5 ± 0.6[†]	5.0 ± 0.4[†]
Triglycerides[**]	105.0 ± 10.3	116.1 ± 11.0	126.2 ± 11.7[†]	137.0 ± 9.8[†]
LDL-cholesterol[**]	79.9 ± 8.0	94.5 ± 14.8	116.8 ± 13.8[†]	126.9 ± 12.2[‖]
LDL:HDL-C[*]	1.9 ± 0.3	1.8 ± 0.4	2.8 ± 0.5[†]	3.2 ± 0.4[¶]
TG:HDL-C[*]	2.2 ± 0.4	2.2 ± 0.3	3.1 ± 0.5[§]	3.5 ± 0.4[†]
Caeruloplasmin[**]	20.5 ± 2.4	20.5 ± 2.2	51.7 ± 2.5[†]	51.1 ± 1.9[†]

[*]Ratio; [†]$p < 0.001$; [††]$p = 1.7609$; [§]$p = 2.53035$; [‖]$p = 1.2743$; [¶]$p = 1.0255$; [**]mg/dl.

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