Myocardial infarction patients show altered lipoprotein properties and functions when compared with stable angina pectoris patients

Kyung-Hyun Cho[1,4], Dong-Gu Shin[2], Suk-Hwan Baek[3] and Jae-Ryong Kim[3]

[1]School of Biotechnology
Aging-associated Vascular Disease Research Center
Yeungnam University
Gyeongsan 712-749, Korea
[2]Cardiovascular Division, Internal Medicine
Yeungnam University Medical Center
Daegu 705-717, Korea
[3]Department of Biochemistry and Molecular Biology
Aging-associated Vascular Disease Research Center
Yeungnam University
Daegu 705-717, Korea
[4]Corresponding author: Tel, 82-53-810-3026;
Fax, 82-53-814-3026; E-mail, chok@yu.ac.kr
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Abstract

Several parameters and risk factors were compared between Korean male myocardial infarction (MI) patients (n = 10) and angina pectoris (AP) patients (n = 17) to search unique biomarkers for myocardial infarction (MI) in lipoprotein level. Individual serum and lipoprotein fractions (VLDL, LDL, HDL_2, HDL_3) were isolated and analyzed by lipid and protein determination and enzyme assay. The MI group was found to have a 25 and 30% higher serum cholesterol and triacylglycerol (TG) level than the AP group, respectively, however, their body mass index (BMI), LDL-cholesterol (C), HDL-C, and glucose levels fell within the normal range. MI patients were found to have an approximately two-fold higher level of serum IL-6 and an 18% lower serum apoA-I level than that of the AP group. LDL and HDL_2 fraction of the MI group were more enriched with TG than those of AP group. The increased TG was correlated well with the increased level of apoC-III in the same fraction. Cholesteryl ester transfer protein (CETP) activity and protein level were greatly increa-

sed in MI patients in the LDL and HDL_3 fractions. MI patients showed more severely oxidized LDL fraction than patients in the AP group, as well as the weakest antioxidant ability of serum. Conclusively, MI patients were found to have unique serum and lipoprotein characteristics including increased IL-6 and TG in serum, with CETP and apoC-III in the LDL and HDL fractions, as well as severely impaired antioxidant ability of HDL.

Introduction

Coronary heart disease (CHD) is responsible for the vast majority of cases of unstable angina, acute myocardial infarction (MI), and sudden death. MI is an acute and catastrophic event that results from acute coronary syndrome (ACS) and atherosclerosis, which chronically progresses to ACS (Thygesen *et al*., 2007). Myocardial infarction is a leading cause of morbidity and mortality in adults in western countries and Asia (Rosamond *et al*., 2007), and diagnostic biomarkers for MI such as serum levels of glutamic oxaloacetic transaminase (GOT), lactate dehydrogenase (LDH), creatine kinase (CK)-MB, and troponin I and T have been developed and utilized clinically (Jaffe *et al*., 2000). However, the aforementioned markers are limited because they provide insufficient specificity for predictive MI diagnosis. Additionally, although several biomarkers have been reported to predict cardiovascular risk, there have been no useful biomarkers developed for the early detection of MI (Pahor *et al*., 1999). Inflammation is a major risk factor of atherosclerosis, and several inflammatory biomarkers such as high sensitivity C-reactive protein (hsCRP), cytokines, biomarkers of endothelial cell activation, and proinflammatory enzymes have been established for the diagnosis of ACS (Hartford *et al*., 2007); however, no studies have been reported to determine if these biomarkers are useful for the prediction of acute MI.

There have been many noninvasive biochemical measures used to predict cardiovascular risk, such

as lipid and lipoprotein metabolism, inflammation, and oxidative stress (Tsimikas, 2006; Thygesen et al., 2007; Walldius et al., 2007). However, it is necessary to develop more readily available specific and prognostic biomarkers that allow MI to be distinguished from AP. Because MI is a major cause of sudden death and disability worldwide, rapid assays for the early detection of MI would have widespread application. However, to date, there have been no studies conducted to compare the characteristics of serum and lipoprotein levels of AP and MI patients.

In the current study, the serum parameters, lipoprotein properties, and lipoprotein functions of Korean male MI patients, angina pectoris (AP) patients and sex and age matched normal subjects were compared to determine if distinguished difference could be used for the prognostic biomarkers of MI.

Results

Serum lipid profile and inflammatory markers

As shown in Table 1, patients in the MI group were found to have significantly higher levels of serum total cholesterol (TC) and triglyceride (TG) than patients in the other groups, although all patients were found to have a similar BMI. In addition, all participants were found to have similar levels of LDL-C, HDL-C, and glucose, with the exception of those in the MI group, which had the lowest percentage of HDL-cholesterol. Additionally, the serum apoA-I level was significantly decreased by up to 18% in the MI group when compared with AP group, indicating that decreased apoA-I can be a specific marker for ACS, especially in cases of MI.

Inflammatory profiles (Table 2) revealed that patients with AP and MI had hsCRP levels up to

Table 1. Serum lipid profiles of patients and controls.[1]

Group	Angina pectoris (n = 17)	Myocardial infarction (n = 10)	Control (n = 7)
Age (yr)	60.6 ± 9.8	54.5 ± 10.8	50 ± 6
BMI (kg/m^2)	23.5 ± 2.5	23.9 ± 2.3	23.8 ± 2.5
TC (mg/dl)	157 ± 33[2,a]	208 ± 20[b]	168 ± 15[a]
TG (mg/dl)	122 ± 39[a]	175 ± 49[b]	130 ± 23[a]
LDL-C (mg/dl)	106 ± 30	118 ± 35	110 ± 21
HDL-C (mg/dl)	48 ± 8	54 ± 13	51 ± 8
% HDL-C	30 ± 2[a]	25 ± 3[b]	30 ± 3[a]
apoA-I (mg/dl)	148 ± 25[a]	121 ± 21[b]	151 ± 30[a]
Glucose (mg/dl)	149 ± 53	124 ± 18	106 ± 12
CETP (mg/l)	4.0 ± 0.3[a]	4.4 ± 0.4[a]	2.3 ± 0.3[b]

[1]Data were expressed as the mean ± S.D.
[2]Means in the same row not sharing a common superscript are significantly different (P < 0.05) between groups.
BMI, body mass index; CETP, cholesteryl ester transfer protein; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; LPO, lipid hydroperoxide; TC, total cholesterol; TG, triacylglycerol.

Table 2. Inflammatory profiles of serum.[1]

Group	Angina pectoris (n = 17)	Myocardial infarction (n = 10)	Control (n = 7)
MPO (ng/ml)	9.6 ± 4.8[a,2]	9.1 ± 4.6[a]	3.2 ± 0.4[b]
LPO (nmol/ml)	8.2 ± 0.2[a]	8.4 ± 0.1[a]	4.3 ± 0.02[b]
SAA (mg/ml)	60.3 ± 60[a]	51.8 ± 58[a]	6.1 ± 0.5[b]
IL-6 (pg/ml)	6.6 ± 5.4[a]	15.6 ± 4.1[b]	1.9 ± 0.1[c]
hsCRP (mg/dl)	0.7 ± 0.5[a]	0.8 ± 0.4[a]	0.2 ± 0.05[b]
GOT (U/L)	23 ± 5[a]	117 ± 103[b]	24 ± 4[a]
GPT (U/L)	28 ± 13	37 ± 29	33 ± 18
LDH (U/L)	350 ± 66[a]	728 ± 249[b]	422 ± 62[a]
Troponin I (ng/ml)	0.04 ± 0.03[a]	30 ± 13[b]	-
Troponin T (ng/ml)	0.01 ± 0.01[a]	4.9 ± 2.8[b]	-
CK-MB (ng/ml)	1.2 ± 0.5[a]	176 ± 156[b]	-

[1]Data were expressed as the mean ± S.D.
[2]Means in the same row not sharing a common superscript are significantly different (P < 0.05) between groups.
CK-MB, creatine kinase MB; GOT, glutamic oxaloacetic transaminase; GPT, gamma-glutamic pyruvic transaminase; IL-6, interleukin-6; LDH, lactate dehydrogenase; LPO, lipid hydroperoxide; MPO, myeloperoxidase; SAA, serum amyloid A.

4-fold higher than control patients, indicating that the hsCRP level can be a marker for coronary artery disease (CAD), but that it is less specific for MI detection. As expected, when compared to patients in the other groups, patients in the MI group had a significantly higher level of markers already established for MI such as CK-MB, troponin I and T, GOT, and LDH. Interestingly, patients in the MI group were found to have the highest level of serum IL-6, 2-fold more than that of AP group, indicating that IL-6 may be a prominent biomarker for MI. However, the serum levels of myeloperoxidase (MPO), lipid hydroperoxide (LPO), and serum amyloid (SAA) were significantly higher in patients in the AP and MI group than the control group, indicating that those parameters may be biomarkers for CAD, but not for MI. Furthermore, there was no difference in the SAA levels of patients in the AP and MI groups, even though the SAA level of both of these groups was found to be 10-fold higher than that of the control. Moreover, the serum LPO level was approximately 2-fold higher in patients in the AP and MI group than those in the control group, indicating that the LPO level can be a specific marker for CAD including ACS. Taken together, these results indicate that increased serum TG and IL-6 levels can be used as specific biomarkers for the detection of MI in addition to the traditional biomarkers for MI such as troponin and CK-MB level (Table 2).

TG and apoC-III were increased in lipoproteins from MI

The lipoprotein levels were evaluated by determining the cholesterol, TG, and protein contents of each of the groups. As shown in Table 3, the LDL-C and LDL-TG levels were increased in the MI group, however, no significant differences in the VLDL fraction were observed among the groups. Interestingly, the HDL$_2$-C level was decreased and the HDL$_2$-TG level was higher in the MI group than the AP and control groups, which strongly suggests that an increase in TG in the LDL and HDL fractions could be a highly specific symptom of MI. Furthermore, ELISA-based detection revealed that the apoC-III protein level in the LDL and HDL$_2$ fractions of the MI group was increased by up to 45% when compared with the levels observed in the AP group (Figure 1A). These increases in the level of apoC-III are well correlated with the increased TG levels observed in the serum (Table 1) and lipoprotein of the MI group, especially in the LDL and HDL fractions (Table 3). Additionally, these results are in good agreement with previous

Table 3. Lipid and protein composition of lipoproteins from patients.

	Angina pectoris (n=10)			Myocardial infarction (n = 10)			Control (n = 7)		
	TC (mg/dl)	TG (mg/dl)	TP (g/dl)	TC (mg/dl)	TG (mg/dl)	TP (g/dl)	TC (mg/dl)	TG (mg/dl)	TP (g/dl)
VLDL (mg of lipid/ mg of protein)	143 ± 31 (0.49 ± 0.1)	345 ± 84 (1.19 ± 0.29)	0.29 ± 0.07 (1)	141 ± 35 (0.39 ± 0.09)	330 ± 98 (0.91 ± 0.27)	0.36 ± 0.03 (1)	132 ± 16 (0.48 ± 0.03)	240 ± 159 (0.88 ± 0.37)	0.27 ± 0.03 (1)
LDL (mg of lipid/ mg of protein)	1071 ± 359 (1.72 ± 0.57)	169 ± 57 (0.27 ± 0.09)	0.62 ± 0.13 (1)	1563 ± 453 (2.2 ± 0.5)	245 ± 60 (0.34 ± 0.06)	0.71 ± 0.29 (1)	880 ± 224 (1.39 ± 0.3)	156 ± 128 (0.24 ± 0.04)	0.63 ± 0.18 (1)
HDL$_2$ (mg of lipid/ mg of protein)	70 ± 16 (0.36 ± 0.08)	43 ± 27 (0.22 ± 0.14)	0.19 ± 0.03 (1)	52 ± 6 (0.28 ± 0.03)	71 ± 9 (0.39 ± 0.05)	0.18 ± 0.04 (1)	76 ± 18 (0.42 ± 0.10)	29 ± 9 (0.16 ± 0.05)	0.18 ± 0.05 (1)
HDL$_3$ (mg of lipid/ mg of protein)	71 ± 23 (0.18 ± 0.05)	25 ± 14 (0.06 ± 0.03)	0.39 ± 0.1 (1)	54 ± 12 (0.15 ± 0.03)	17 ± 7 (0.05 ± 0.02)	0.34 ± 0.05 (1)	70 ± 25 (0.2 ± 0.07)	13 ± 13 (0.03 ± 0.03)	0.35 ± 0.1 (1)

studies that have found plasma TG to be a significant predictor of CAD (Hokanson *et al.*, 1996), as well as with the results of studies that have found high levels of apoC-III in the VLDL and LDL fractions to be a strong significant predictor of coronary events (Sacks *et al.*, 2000). Taken together, these results indicate that increased levels of TG and apoC-III are directly connected with the incidence of MI.

Increase of CETP protein and activity

In plasma level, the MI and AP group showed a higher CE-transfer activity from HDL to LDL than the control group (Table 4). As shown in Table 1,

this activity was correlated well with the ELISA based detection, with the serum CETP protein content being increased almost 2-fold when compared to that of the control.

When the lipoprotein levels were considered, the MI group showed the highest HDL_3-CETP activity, but there was no significant difference in the HDL_2-CETP activity between the groups. However, the CETP activity of the LDL fraction was 2-fold higher in the AP and MI group (7-10% CE-transfer) than in the control group (2.7% CE-transfer), indicating that LDL-CETP activity in CAD patients was enhanced.

Patients in the MI group had much more CETP protein in the LDL and HDL_3 fractions than those in
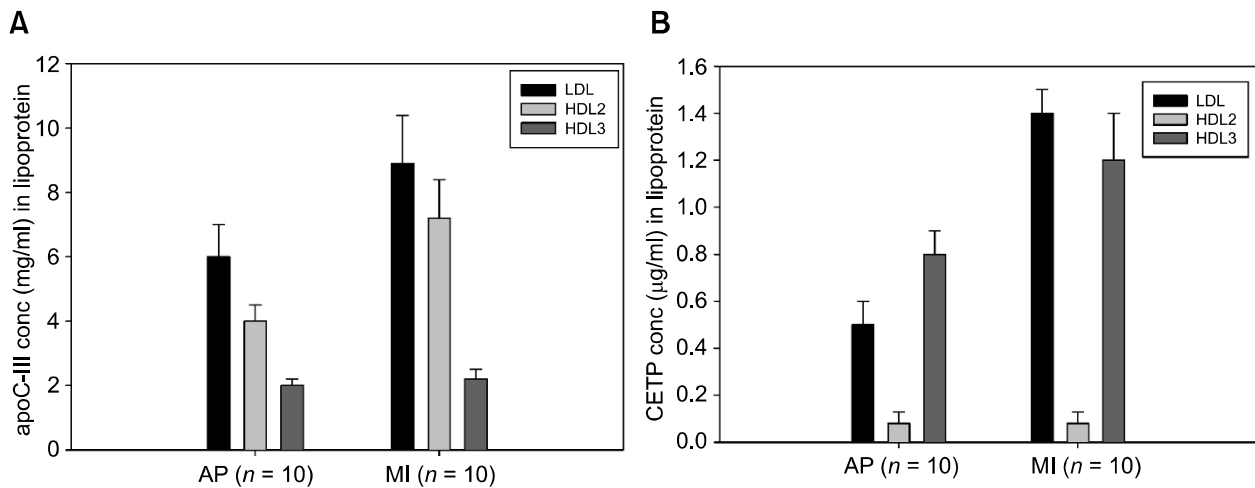
Figure 1. Sandwich ELISA-based detection of apoC-III (panel A) and CETP (panel B) in lipoprotein. Fifty microliters of individual lipoprotein (2 mg of protein/ml) was reacted with polyclonal CETP antibody (Abcam, ab19012) or apoC-III antibody (AB821), which was coated on a 96-well plate. In MI group, protein levels of apoC-III was highly expressed in both the LDL and HDL_2 fraction, while CETP level was increased in LDL and HDL_3 fraction.

Table 4. Lipoprotein-associated enzyme activities.[1]

	Enzyme source	Angina pectoris (n = 10)	Myocardial infarction (n = 10)	Control (n = 7)
CETP	HDL_2	9 ± 5.1	8.9 ± 4.7	8.0 ± 4.6
(% CE transfer from [³H]-	HDL_3	$14.6 \pm 4.7^{a,2}$	25.4 ± 3.6^{b}	10 ± 2^{a}
CE-HDL to LDL/mg of protein)	LDL	7.6 ± 3.3^{a}	10.3 ± 2.5^{a}	2.7 ± 0.1^{b}
	VLDL	3.0 ± 0.4	2.7 ± 0.6	-
	Serum	29.7 ± 8.0^{a}	41.8 ± 6.3^{b}	24 ± 3.3^{c}
PLA2 (pmole PAF/h/mg	LDL	15.5 ± 0.7^{a}	15.3 ± 0.3^{a}	10.3 ± 0.6^{b}
of protein)	HDL_2	9.1 ± 0.5^{a}	11.8 ± 1.3^{a}	29.1 ± 1.3^{b}
	HDL_3	15.0 ± 2.4	16.6 ± 0.9	15.4 ± 1.4
PON (μU/L/mg of protein)	HDL_3	179 ± 69^{a}	113 ± 45^{a}	1671 ± 70^{b}
LCAT (% CE-conversion/hr/ 100 μg of protein	HDL_3	1.8 ± 1.4^{a}	3.1 ± 1.2^{a}	10.2 ± 1.2^{b}

[1]Each assay method was described in text.

[2]Data were expressed as the mean $\pm$ S.D. Means in the same row not sharing a common superscript are significantly different ($P < 0.05$) between groups.

CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; PLA2, phospholipase A2; PON, paraoxonase.

the AP group (Figure 1B) from ELISA based detection, while CETP amount in HDL_2 was not different between the groups. Taken together, apoC-III and CETP levels were significantly co-increased in LDL fraction of MI group (Figure 1).

Enzyme activities of lipoproteins

The paraoxonase (PON) and lecithin:cholesterol acetyl transferase (LCAT) activity in the HDL_3 fraction was several fold lower in the AP and MI groups than the control group (Table 4), suggesting
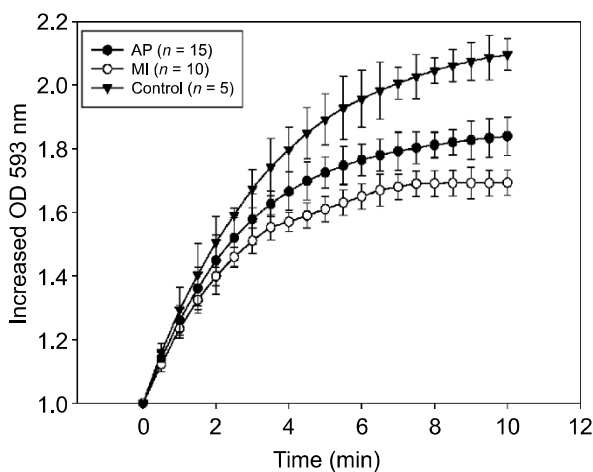

Figure 2. Comparison of the antioxidant ability based on the ferric-reducing ability of plasma (FRAP). The MI group showed the weakest reducing ability among groups, whereas the control group showed the strongest reducing ability after 10 min of incubation.

that the antioxidant ability of HDL_3 was almost completely absent in ACS patients. In addition, the AP and MI groups showed approximately 50% more LDL-associated PLA_2 than the control group. However, HDL_2-associated PLA_2 activity was much lower in the AP and MI groups, being only 63% of that of the control groups. These results are in good agreement with those of a previous report in which LDL-associated PLA_2 activity was found to be an independent risk factor of CAD responsible for triggering the inflammatory process (Packard *et al.*, 2000). Additionally, there was no significant difference in the HDL_3-PLA_2 activity among all groups, indicating that HDL_3-PLA_2 activity was less effected by the onset of the disease. Taken together, the activities of antioxidant enzymes including LCAT and PON were much lower in the AP and MI group than in the control group.

Ferric ion reducing ability of plasma (FRAP)

The FRAP assay is known to be a rapid and reproducible method for determination of the molar concentration of antioxidants present in plasma *in vivo* (Benzie and Strain, 1996). We conducted a FRAP assay using patients' sera to compare the *in vivo* antioxidant effects among groups. As shown in Figure 2, the MI group showed only a 69 $\pm$ 4% increase in absorbance at 593 nm (A593) during 10 min of incubation, while the AP and control group showed 83 $\pm$ 6% and 109 $\pm$ 5% increases, respectively. This result indicates that the FRAP ability of the MI group was lower than that of the AP group, indicating the weakest antioxidant ability.

A

B
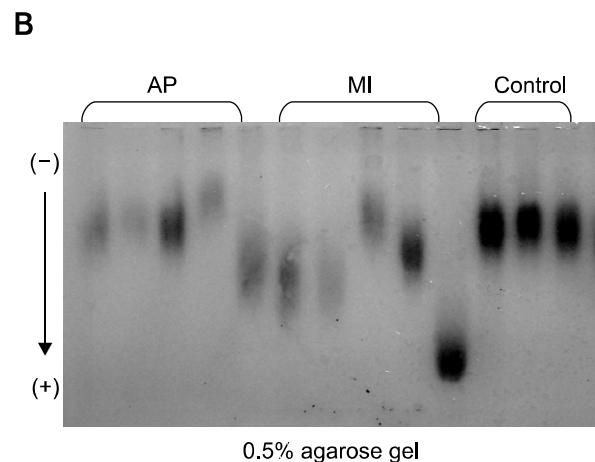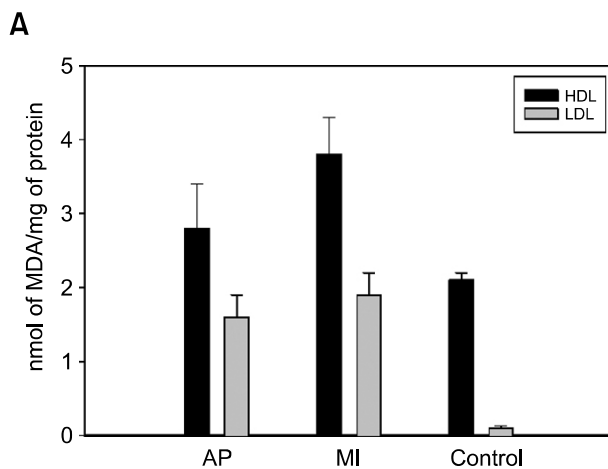
0.5% agarose gel

Figure 3. TBARS assay of LDL without copper mediated oxidation (panel A) compared with the malondialdehyde content and electromobility in agarose gel (panel B) for patients and controls. MI patients showed the highest level of MDA content and the fastest electromobility on the agarose gel. Copper-mediated oxidation of HDL_3 (panel A) from patients and controls. Each HDL_3 (50 μl, 2 mg/ml) was incubated with $CuSO_4$ (final 25 μM) for 5 h at 37°C.

More oxidized LDL was detected in MI patients

The extent of LDL oxidation among patients and control was compared. Based on the results of TBARS analysis, the LDL of MI and AP patients contained significantly higher levels of MDA (1.9 ± 0.3 and 1.6 ± 0.7 nmol, respectively) than that of the control group (0.1 ± 0.03 nmol) (Figure 3A). In addition, an electrophoretic mobility assay confirmed that the LDL fraction of MI and AP patients migrated much faster with more decayed band intensity than that of the control group (Figure 3B). Taken together, these results strongly suggest that the LDL of MI patients is more oxidized than that of AP patients, even though the LDL of AP patients had a higher TC and TG content (Table 3). These findings are in agreement with those of a previous report that found oxLDL levels to be significantly positively correlated with the severity of acute coronary syndromes (Ehara *et al.*, 2001).

Copper mediated oxidation of HDL

Copper mediated oxidation was conducted with HDL_3 obtained from the patient and control groups. HDL_3 (2 mg of protein/ml) from the MI group showed the most sensitivity to oxidation, with production of 3.8 ± 0.5 nmol MDA being observed in response to treatment with 25 μM copper as opposed to only 2.1 ± 0.1 nmol MDA being produced by the same treatment in the control group (Figure 3A). This result suggests that the antioxidant ability of HDL from the MI group was more impaired than that of HDL obtained from the AP group.

Discussion

Although MI and AP both have the same pathological mechanism for development, their clinical symptoms and prognosis are different. Numerous studies have suggested that there are several biomarkers for the diagnosis of MI; however, no unique prognostic biomarkers for early detection of MI or ischemic heart disease progress have been identified. Furthermore, it is difficult and inconvenient to distinguish MI from AP due to an invasive protocol. Therefore, this study was conducted to identify unique prognostic biomarkers to distinguish MI and AP in the serum and serum lipoprotein level.

Even though the number of MI patients in the current study was small ($n = 10$), the findings of this study should not be underestimated because the patients were first diagnosed as MI without co-morbid diseases did not take any other medication including statins. The MI patients showed typical diagnostic characters of the Asian-Pacific population, including not being obese and having normal level of TC (approx. 208 ± 20 mg/dl) and HDL-C (approx. 54 ± 13 mg/dl). Those numbers are fall in normal ranges suggested by the national cholesterol education program-adult treatment panel (NCEP-ATP) III guidelines. This indicates that simple application of the NCEP guideline might be limited in its ability to identify the risk of MI for Asian-Pacific patients. It has been speculated that there may be an abrupt increase of inflammatory markers and oxidative stress in the serum of MI patients. The oxidative modification of LDL, which is considered to be a strong risk factor for atherosclerosis and ACS, occurs through the release of a proinflammatory and oxidative signal. These information are confirmed by the results of the current study, which indicated that patients in the MI group contained more oxidized LDL and increased serum IL-6 levels than patients in the other groups. In addition, the serum MPO and LPO levels were co-increased in the AP and MI group, which indicate that increased oxidative stress is a fundamental power of ACS. Serum MPO is involved in an inflammatory process that leads to the production of dysfunctional HDL through oxidative modification (Shao *et al.*, 2006; Meuwese *et al.*, 2007). However, it is difficult to distinguish MI patients from AP patients based on hsCRP levels because the levels are similar in both types of patients (Table 2). This suggests that hsCRP may not be a unique biomarker for the detection of MI, although it is a good diagnostic marker for detection of CAD. A number of population based study have suggested that there is a strong consistency in the relationship between ACS and inflammatory markers, such as hsCRP (Tuomisto *et al.*, 2006), IL-6, and lipoprotein associated phospholipase A_2 (Ballantyne *et al.*, 2004). These markers have been elevated in CVD patients and frequently precede to the development of ACS. Our current study also supports the same increase of the markers; serum IL-6 level, LDL-PLA_2 activity, and white blood cell number were significantly increased in AP and MI group.

A low HDL-C level is the most common lipid abnormality observed in families with premature CHD (Genest *et al.*, 1992). However, in the current study, the MI group was found to have a slightly higher level of serum HDL-C than the AP group, even though MI patients were found to have a lower percentage of HDL-C per TC than AP patients (Table 3). This result suggests that a simple comparison of serum HDL-C levels might not be useful for the prediction of MI. More importantly, HDL_2-C level was lower and HDL-TG level was higher in MI patients than that of AP patients,

indicating that the lipid composition of HDL_2 might be useful for evaluating the risk of MI. Similarly, we recently reported that there is a direct relationship between an increase in the levels of apoC-III, serum TG, acute inflammatory process markers such as IL-6, and a decrease of HDL-C in the oliguric phase of hemorrhagic fever renal syndrome (HFRS) patients (Cho *et al.*, 2008). In addition, Hartford *et al.* (2007) reported that CRP, IL-6, and sPLA2 are intercorrelated in patients with ACS, whereas iCAM-1 appears to respond differently to acute coronary events. Recently Suzuki *et al.* (2008) reported that elevation of matrix metalloproteinases and IL-6 are involved in the instability of vulnerable plaque associated with the induction of myocardial infarction. Taken together, these results strongly indicate that a decrease in HDL-C and an increase in HDL-TG are linked to a malfunction of HDL, which is responsible for the antioxidant power against oxidation of LDL.

Many studies have suggested that the TG-rich lipoproteins are proatherogenic (McBride, 2007; Libby, 2007). The presence of increased TG in the lipoprotein is in good agreement with the results of previous studies that indicated that TG levels are an important and independent predictor of CHD and stroke risk in the Asia-Pacific region (Asia pacific cohort studies collaboration, 2004). Furthermore, the serum TG was accumulated more in the LDL and HDL_2 fractions than in the VLDL fraction, which is similar to the results of previous studies that have found a strong and consistent association of hypertriglyceridemia with a dense LDL fraction (Austin *et al.*, 1988; Campos *et al.*, 1992). In this study, MI patients were found to have higher level of apoC-III, CETP, and TG-enriched LDL that was more oxidized than AP group.

Overall, the results of this study revealed that MI patients showed significantly increased serum IL-6 and TG levels and decreased serum apoA-I levels when compared to AP patients. Levels of the TG, apoC-III and CETP in the LDL and HDL fractions of MI patients were increased, whereas the level of HDL-C was decreased. These results strongly suggest that the composition of lipoprotein is altered in MI patients with more increase of inflammatory factors, even though the levels of oxidative species were similar among AP and MI patients.

Methods

Patients and controls

Stable AP patients (n = 17) were selected using the following criteria: the presence of chest or arm discomfort that is rarely described as pain, but is reproducibly associated with physical exertion or stress and relieved within 5 to 10 min of rest and/or administration of sublingual nitroglycerin. The diagnosis was confirmed with treadmill exercise test and coronary angiography in all patients.

MI patients (n = 10) were defined as individuals that exhibited angina pectoris or equivalent ischemic discomfort and at least one of the following three features: [1] symptoms occur at rest (or with minimal exertion) and usually last > 10 min, [2] symptoms are severe and of new onset, and/or [3] symptoms exhibit a crescendo pattern (i.e., becoming distinctly more severe, prolonged, or frequent with time) (Kasper *et al.*, 2005). In addition, the presence of any one of the following criteria when there was evidence of myocardial necrosis in a clinical setting consistent with myocardial ischemia was diagnosed as myocardial infarction: [1] Electrocardiogram (ECG) changes indicative of new ischemia (new ST-T changes or new left bundle branch block), [2] development of pathologic Q waves in the ECG, and [3] imaging evidence of new loss of viable myocardium or new regional wall motion abnormality (Thygesen *et al.*, 2007).

All AP and MI patients were first diagnosed, and patients with diabetes, history of familial dyslipidemia and previous use of medications including statin, were excluded from the analysis. Percutaneous coronary interventions were performed at the physician's discretion if needed, as indicated in the guideline. Patients did not take any medication besides statin prior to hospitalization.

Age and gender matched reference subjects (n = 7) were recruited from healthy volunteers who visited Health Center of Yeungnam University Hospital (Daegu, Korea) for regular health check up. They had unremarkable medical records without history of endocronological disorder.

Informed consent was obtained from all patients and reference individuals (control group) prior to enrollment in the study and the institutional review board at the Medical Center of Yeungnam University approved the protocol.

Plasma and lipoprotein

Blood was drawn on overnight fasting state in AP patients, and blood sampling was done in the emergency room immediately after the diagnosis of MI was confirmed by a detailed clinical history, ECG and/or serologic tests in case of MI patients. Blood was collected using a vacutainer (BD sciences, Franklin Lakes, NJ) containing EDTA (final 1 mM). Plasma was isolated by low speed centrifugation and stored at -80°C until analysis.

VLDL (d < 1.019 g/ml), LDL (1.019 < d < 1.063), HDL_2 (1.063 < d < 1.125), and HDL_3 (1.125 < d < 1.225) were isolated from individual patient and control serum via sequential ultracentrifugation, with the density being appropriately adjusted by the addition of NaCl and NaBr in accordance with standard protocols. Samples were centrifuged for 24 h at 10°C at 100,000 *g* using a Himac CP-90α (Hitachi, Tokyo, Japan) at the Instrumental Analysis Center at Yeungnam University.

Biochemical analysis

Blood parameters, lipids, and glucose concentrations were

determined using an automatic blood analyzer (Chemistry analyzer AU4500 Olympus, Tokyo, Japan). For each of the lipoproteins (VLDL, LDL, HDL_2, HDL_3) which was purified individually, total cholesterol (TC) and triglyceride (TG) measurements were obtained using commercially available kits (cholesterol, T-CHO; triglyceride, Cleantech TS-S, Wako Pure Chemical, Osaka, Japan). The protein concentrations of lipoprotein were determined via Lowry protein assay, as modified by Markwell *et al.* (1978) and using Bradford assay reagent (BioRad, Seoul, Korea) with BSA as a standard. The blood levels of the MPO were determined using an ELISA kit that was obtained from Immunology Consultants Lab (Cat # E-80PX, Newberg, OR). Serum lipid hydroperoxide (LPO) was determined colorimetrically using a commercial kit (Cat# 437639, Calbiochem, San Diego, CA) in accordance with the manufacturer's suggestions.

Ferric reducing ability of plasma assay

The ferric reducing ability of plasma (FRAP) was determined using the method described by Benzie and Strain (1996). Briefly, the FRAP reagents were freshly prepared by mixing 25 ml of 0.2 M acetate buffer (pH 3.6), 2.5 ml of 10 mM 2,4,6, tripyridyl-s-triazine (TPTZ, Fluka Chemicals), and 2.5 ml of 20 mM $FeCl_3$ $6H_2O$. The antioxidant activities of the serum were then estimated by measuring the increase in absorbance induced by the generated ferrous ions. The freshly prepared FRAP reagent (300 μl) was mixed with equally diluted serum (20 mg/ml, 10 μl), after which the ferric reducing ability was determined by measuring the absorbance at 593 nm every 20 s over a 10 min period at 25°C using a DU800 spectrophotometer (Beckman Coulter, Fullerton, CA) equipped with a Multi-iTemp III thermocirculator (Amersham, Uppsala, Sweden).

Cholesteryl ester conversion assay

Cholesteryl ester conversion was conducted via lecithin: cholesterol acyltransferase (LCAT) assays as previously described (Han *et al.*, 2005). Serum (50 μl) or HDL fractions (28 μg of protein in 30 μL) were utilized as the enzyme source. ApoA-I-rHDL containing radiolabeled cholesterol (1 μCi of [^{14}C]-4-cholesterol/69 μg of cholesterol/1 mg of apoA-I) was employed as a substrate, and the apoA-I was then expressed using an *E. coli* expression system as described previously (Cho *et al.*, 2006). Discoidal rHDL was prepared via the sodium cholate dialysis method using initial molar ratios of palmitoyloleoyl phosphatidylcholine (POPC)-cholesterol-apoA-I-sodium cholate at a ratio of 95:5:1:150 (wt/wt/wt/wt).

The reaction was initiated via the addition of individual serum, and the mixture was then incubated for 1 h at 37°C. Next, the esterified cholesterol and free cholesterol were separated via TLC, and the activity was expressed as the % conversion rate of cholesteryl ester from free cholesterol.

Paraoxonase assay

Paraoxonase-1 (PON-1) activity toward paraoxon was determined by evaluating the hydrolysis of paraoxon into

p-nitrophenol and diethylphosphate, which was catalyzed by the enzyme (Eckerson *et al.*, 1983). PON-1 activity was then determined by measuring the initial velocity of *p*-nitrophenol production at 37°C, as determined by measuring the absorbance at 405 nm (Microplate reader, Bio-Rad model 680). To accomplish this, an equal amount of serum (10 μl) was added to 200 μl of substrate (paraoxon-ethyl, Sigma D-9286)-containing solution (90 mM Tris-HCl/3.6 mM NaCl/2 mM $CaCl_2$, pH 8.5). The PON-1 activity of 1 U/L was defined as 1 μmol of *p*-nitrophenol formed per minute. The molar extinction coefficient of *p*-nitrophenol was 17,000 M^{-1}, cm^{-1}.

Cholesteryl ester transfer assay

An rHDL containing apoA-I and cholesteryl oleate was synthesized in accordance with the method described by Cho *et al.* (1998) using trace amounts of [^{3}H]-cholesteryl oleate (GE healthcare, TRK886, 3.5 μCi/mg of apoA-I). Next, to facilitate separation from the CE-acceptor, the rHDL was immobilized using a CNBr-activated Sepharose 4B resin (Amersham Biosciences, Uppsala, Sweden) in accordance with the manufacturer's instructions. The CE-transfer reaction was allowed in 300 μl reaction mixtures that contained each of the serum samples (20 μl) as a CETP source and rHDL-agarose (50 μl, 0.25 mg/ml) and human LDL (50 μl, 0.25 mg/ml) as a CE-donor and CE-acceptor, respectively. After incubation at 37°C, the reaction was halted via brief centrifugation (10,000 *g*) for 3 min at 4°C. The supernatant (150 μl) was then subjected to scintillation counting, and the percentage transfer of [^{3}H]-CE from rHDL to LDL was calculated.

Lipoprotein-associated phospholipase 2 assay

The individual lipoprotein fraction (10 μl, 20 μg) was used as an enzyme source for the PLA_2 reaction, with an Lp-PLA_2 assay being conducted according to the method described by Boyd *et al.* (2000). Briefly, [^{3}H]-platelet activating factor (Hexadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine, Perkin-Elmer, NET910, 0.1 mCi/ml) and 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine was used as a substrate for the reaction. Substrate solution containing 10 μl of the [^{3}H]-PAF (1 μCi, 50 μM) and 12 μM of cold PAF was incubated using each lipoprotein solution as an Lp-PLA_2 source for 30 min. The reaction was then stopped by vortexing the solutions with 600 μl of $CHCl_3$: Me-OH (2:1, v/v), after which the aqueous layer (150 μl) was removed. The aqueous layer was then vortexed again with $CHCl_3$, after which it was centrifuged and the upper phase was used for scintillation counting.

ELISA, western blot analysis, and electrophoresis

For sandwich ELISA, diluted primary antibody (1:2,000 or 1:4,000) was coated onto a 96 well plate (Nunc Maxisorp 439454) overnight at 4°C. As primary antibodies, apoA-I antibody (Abcam, ab7613) and CETP antibody (ab19012) were purchased from Abcam (Cambridge, UK), and apoC-III antibody was obtained from Chemicon (Cat# AB821, Temecula, CA). HRP-conjugated apoA-I antibody

(ab20784) and HRP-apoC-III antibody (ab27624) were purchased from Abcam and used as secondary antibodies. A substrate reagent pack (R&D system, DY999) was used for color development.

The apolipoprotein/lipoprotein compositions were compared via SDS-PAGE with an equal amount of protein loading, and the expressional level of apolipoprotein was analyzed via Western blot analysis. In order to compare the electromobility of patient and control samples, the migration of each lipoprotein (LDL, HDL$_2$, and HDL$_3$) was evaluated by agarose electrophoresis. The gels were then dried and stained with 0.125% Coomassie brilliant blue, after which the relative band intensities were compared via band scanning using a Gel Doc XR (Bio-Rad, Hercules, CA) with Quantity One software, version 4.5.2.

Data analysis

All data were expressed as the mean $\pm$ S.D. The data were evaluated via one-way analysis of variance (ANOVA) using an SPSS program (version 12.0, Chicago, IL), and the differences between the means were assessed using Duncan's multiple-range test. Statistical significance was defined as $P < 0.05$.

Acknowledgements

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