

Association between HDL Particles Size and Myeloperoxidase/ Paraoxonase-1 (MPO/PON1) Ratio in Patients with Acute Coronary Syndrome

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Abstract- Myeloperoxidase (MPO) and paraoxonase-1 (PON1) are inflammatory and anti-inflammatory enzymes, respectively that have been involved in the pathogenesis of coronary artery disease (CAD). In this study we sought to evaluate the relations of MPO and PON1 with high density lipoprotein (HDL) mean size in patients with acute coronary syndrome (ACS). Collectively, 50 control subjects and 50 patients with ACS were participated in this study. MPO level and PON1 activity was determined using immunoassay and colorimetric methods, respectively. HDL mean size was determined by a dynamic light scattering methodology. Other clinical risk factors were also determined by standard methods. The MPO/PON1 ratio amount was significantly higher in patients with ACS (1.49 ± 1.10) than in control subjects (0.21 ± 0.14) ($P < 0.01$). There was a significant correlation between MPO/PON1 ratio and HDL mean size in patients with ACS. Amount of the enzymes and their relations to HDL particle size in patients with ACS may play a part in the pathogenesis of ACS. Also, MPO/PON1 ratio may be a robust predictor of ACS.

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Introduction

Coronary artery disease (CAD) is a major cause of morbidity and mortality throughout the world (1). Inflammation and oxidative stress have been implicated as key events in the pathogenesis of CAD (2,3). Myeloperoxidase (MPO) is an inflammatory and oxidative related stress enzyme which is released by activated neutrophils and macrophages (4) and is involved in the initiation, progression and complication of CAD and thus is known as an independent risk factor for CAD (5). On the other hand, high density lipoprotein (HDL) is known to protect against atherosclerosis through antioxidative and antiatherogenic mechanisms (6,7). Paraoxonase-1 (PON1) is also an antioxidant and antiatherogenic enzyme that predominately associated with HDL particles (8) and it is suggested that some antiatherogenic capacity of HDL is related to the

associated enzyme, PON1 (9).

It has been demonstrated that MPO involves in HDL dysfunction. MPO diminishes the capacity of HDL to remove cholesterol from cells and impairs its binding to the scavenger receptor B-1 (SR-BI) (10,11). HDL has some beneficial effects on endothelium and it has been shown to prevent low density lipoprotein (LDL) oxidation through the removal of oxidized phospholipids (6). Altogether, PON1 hydrolyzes oxidized phospholipids on LDL and HDL particles thereby could decrease the risk of vascular disease (11). Recent studies have suggested that PON1 may be particularly sensitive to the oxidation conditions that are established by MPO enzymatic activity (12).

It has been reported that increased HDL particle size may lead to its dysfunction (13). Some evidence revealed that the small and dense subgroups of HDL (HDL₃) possess a higher capacity to protect LDL against oxidation than the larger and light HDL subgroups

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(HDL₂) (14,15). It has also been shown that *in vitro* oxidation of HDL is affected by its size and composition (16). Both the particle size and the concentration of HDL are independently related to the risk of developing CAD (17). PON1 activity is reported to be associated predominately with the HDL₃ particle (18). Furthermore, it is appeared that HDL₂ particles are more prone to oxidation than HDL₃ particles (16).

Given the importance of relationships between HDL size and function, it is seemed that MPO and PON1 to be the important determinants of HDL function. The aim of this study was to evaluate the relationships between MPO level and PON1 activity with HDL particle size in patients with ACS. The reciprocal interactions between HDL particle size and MPO level or PON1 activity in ACS, as the severe presentation of CAD remain to be revealed. We hypothesized that amounts of the two enzymes, especially their ratio, in relation with HDL particle size and some cardiovascular risk factors in patients with ACS in comparison with control subjects, may better reveal their roles in CAD pathogenesis and may have clinical application.

Materials and Methods

Subjects

From subjects who consecutively referred to Isfahan Cardiovascular Research Center in Iran, fifty patients with ACS and fifty control subjects were selected for the study. All subjects were diagnosed angiographically following suspicious clinical presentations of cardiovascular diseases. Patients with ACS had a coronary artery stenosis $\geq 50\%$ in at least one of their three main coronary arteries. The patients also had an unpredictable chest pain at rest or sleep in addition to a transient ST-segment shift. In control subjects, coronary artery obstruction was less than 50%. In fact, the majority (86%) of the control subjects had no obvious stenosis. The information about customary risk factors and medication were acquired via standardized questionnaire after admission. Hypertension was approved when the systolic blood pressure ≥ 140 mm Hg and diastolic blood pressure ≥ 90 mm Hg on two consecutive times or the patients consume antihypertensive drugs. Diabetes mellitus was assigned as a fasting serum glucose ≥ 126 mg/dl and hemoglobin A1c $\geq 6.5\%$, or use of hypoglycemic medication. Family history of CAD was validated as CAD events in first ranked relatives at < 55 years of age in men or < 65 years in women. The use of any form of tobacco and cigarette in any quantity were translated into smoking.

Other clinical information such as body mass index (BMI) and life style were also provided.

Patients with any recent cardiovascular events, surgery, cancer, and chronic or acute inflammatory diseases were not included in this study. After angiography, patients with any other complicated conditions such as angioplasty and myocardial infarction were also excluded from the study. Written informed consent was signed by each subject and the study was confirmed by the ethics committee of the center.

Biochemical analysis

One week after angiography subjects were referred to the Center for sampling. Blood sample of each subject was collected by venipuncture after 12-14 hours fasting. Serum and EDTA-plasma samples were separated by centrifugation at 3000 rpm for 15 minutes. The aliquots of serum and plasma (each about 200 μ l) were frozen at -80°C until the accomplishment of the assays.

Lipid profile including total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol were measured via enzymatic methods by using of commercial kits (DiaSys Diagnostic Inc., Holzheim, Germany) on an automated analyzer (Hitachi 902, Japan). Accordingly, high sensitive C reactive protein (hsCRP) was determined by an immunoturbidometric method by using of a commercial kit (DiaSys Diagnostic Inc., Holzheim, Germany).

Determination of serum PON1 activity

PON1 activity was measured using 2 mM paraoxon (diethyl p-nitro-phenyl phosphate, Sigma chemical Co., USA) as the substrate in 100 mM tris buffer, pH 8.0, containing 2 mM of CaCl_2 . A 40 μ l aliquot of serum was added to 500 μ l of the substrate medium and the initial rate of hydrolysis (generation of p-nitrophenol) was determined at 412 nm using UV-VIS-3100 spectrophotometer (Shimadzu, Kyoto, Japan) over a period of 2 min. The molar extinction coefficient of p-nitrophenol was considered 17,000 M/cm (19). All assays were performed in duplicate at 25°C . The intra-assay and inter-assay coefficient of variation (CV) for the method was shown to be 2.9% and 6.3%, respectively.

Determination of plasma MPO level

The plasma level of MPO was measured by an immunoassay kit (Immunology Consultants Laboratory, Inc., Newberg, USA). The intra-assay and inter-assay CV% of the kit were 2.8% and 6.5%, respectively.

HDL mean size measurement

HDL mean size was determined by the method similar to that reported by Emerson except in that instead of laser light scattering (LLS), dynamic light scattering (DLS) methodology was used (20). To do this experiment, after thawing of the EDTA-plasma samples at room temperature, polyethylene glycol (PEG) 8000 (Scharlau, Chemie S.A., Barcelona, Spain) was added and mixed to precipitate apo B containing lipoproteins. Therefore, 0.5 ml of plasma was mixed with 0.5 ml of PEG solution (400 g/l in 0.2 mol/l glycine buffer adjusted to pH 10 with sodium hydroxide) and vortexed for 30 seconds. Then, after centrifugation at 1800 x g for 10 min at 25°C, 0.5 ml of the supernatant was mixed gently with 1.5 ml of NaCl (10 mM) and passed through a syringe filter (cellulose acetate membrane, 30 mm, pore size: 0.2 µm, Orange Scientific). The filtrate was injected into a disposable polystyrene cell (DTS0012) and subjected to size determination by a Zetasizer Nano ZS Instrument (Malvern, Worcestershire, UK) with a 532 nm green laser beam. The scattered light was collected by a detector at the angle of 173° using NIBS (Non-Invasive Back-Scatter) technology and directed to a correlator. The data was analyzed by Zetasizer software (DTS, nano series, version 5.02, Malvern, and Worcestershire, UK) and size information was reported as the Z-Average by intensity (21,22). All measurements were performed at 25°C, in duplicate with automatic duration measurements.

The accuracy of size measurements was examined using standard size nano particles (Gold Nanoparticles, 20 nm, 0.01% (w/v) aqueous solution, Nanocs Inc, NY, USA) under the same experimental conditions and the

results were matched with the diameter quoted by the manufacturer. The within assay coefficient of variation (CV) for 10 measurements was 1.2% and the between assay CV for 8 measurements was 3.1%.

Statistics

Statistical analysis was performed using of SPSS 16.0 (SPSS Inc., Chicago, IL). Data with normal distribution were expressed as means ± SD. Logarithmic conversion was conducted on skewed variables. Chi-square test was used to compare dichotomous variables. Pearson's correlation coefficient analysis was used to calculate the correlation between variables with normal distribution.

Results

Clinical characteristics data of all subjects are summarized in table 1. Patients and control subjects were age and gender matched. As seen in the table, cardiovascular risk factors were higher in patients than in control subjects. In patients with ACS the biomarkers, hsCRP and MPO, were profoundly higher than in control subjects but serum PON1 activity was significantly lower. Although lipid profiles were higher in patients with ACS than in control subjects, these differences was negligible except for LDL-cholesterol that was high enough in patients with ACS in comparison with control subjects ($P=0.01$). The MPO/PON1 ratio was also significantly higher in patients with ACS than in control subjects (Table 1).

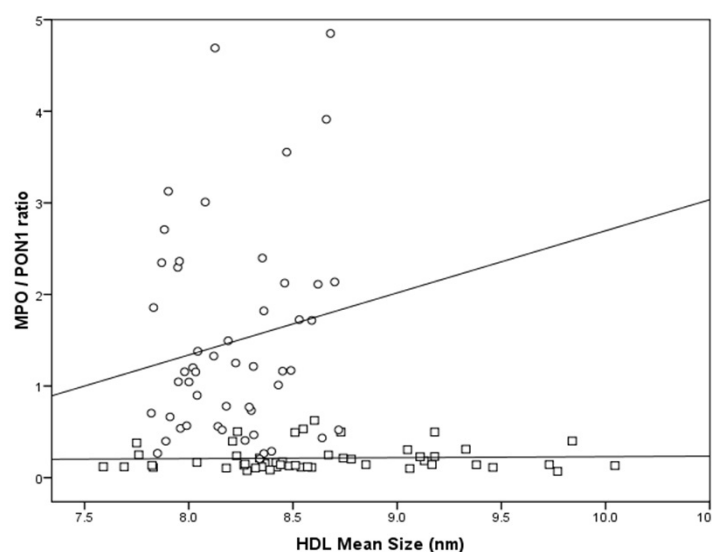


Figure 1. The correlation between MPO/PON1 ratio and HDL mean size in control subjects (□) and patients with ACS (○).

Table 1. The clinical Characteristics of patients.

Variable	Control subjects (n=50)	Patients with ACS (n=50)	P-value
Age (year)	56.96 ± 8.30	55.78 ± 11.68	0.56
Men	35 (70%)	32 (64%)	0.40
BMI (kg/m ²)	25.90 ± 0.71	25.79 ± 2.32	0.48
Total Cholesterol (mg/dl)	178.84 ± 47.00	192.93 ± 36.31	0.07
Triglyceride (mg/dl)	147.42 ± 97.30	159.89 ± 80.40	0.08
LDL (mg/dl)	96.73 ± 25.54	109.40 ± 23.79	0.01
HDL (mg/dl)	42.53 ± 4.49	44.38 ± 9.51	0.12
hsCRP (mg/l)	2.89 ± 1.31	4.92 ± 1.68	< 0.01
MPO (ng/ml)	21.93 ± 3.58	71.22 ± 19.54	< 0.01
MPO/PON1	0.21 ± 0.14	1.49 ± 1.10	< 0.01
HDL mean size (nm)	8.2 ± 0.3	8.6 ± 0.6	< 0.01
PON1 (U/l)	138.73 ± 71.18	76.81 ± 59.37	< 0.01
Hypertension	3 (6%)	20 (40%)	< 0.01
Diabetes mellitus	1 (2%)	20 (40%)	< 0.01
Family history of CAD	10 (20%)	30 (60%)	< 0.01
Smoking	2 (4%)	15 (30%)	< 0.01
Aspirin	0 (0%)	21 (42%)	< 0.01

Data are expressed as mean ± SD or number (%); BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; hsCRP, high sensitive C-Reactive Protein; MPO, myeloperoxidase; PON1, paraoxonase-1; CAD, coronary artery disease.

The relationships between MPO/PON1 ratio and some of the clinical biomarkers are shown in table 2. MPO/PON1 ratio demonstrated a significant association with age, BMI, hsCRP and HDL size, but was not correlated with lipid profile (Table 2).

The association between MPO/PON1 ratio and HDL mean size is demonstrated in figure 1. As seen, the

correlation was significant only in patients with ACS ($r=0.31$, $P=0.03$).

We did not find a significant correlation between paraoxonase and HDL particle size ($r=0.09$, $P=0.38$), but the correlation between paraoxonase and hsCRP was inversely significant ($r=-0.30$, $P=0.03$).

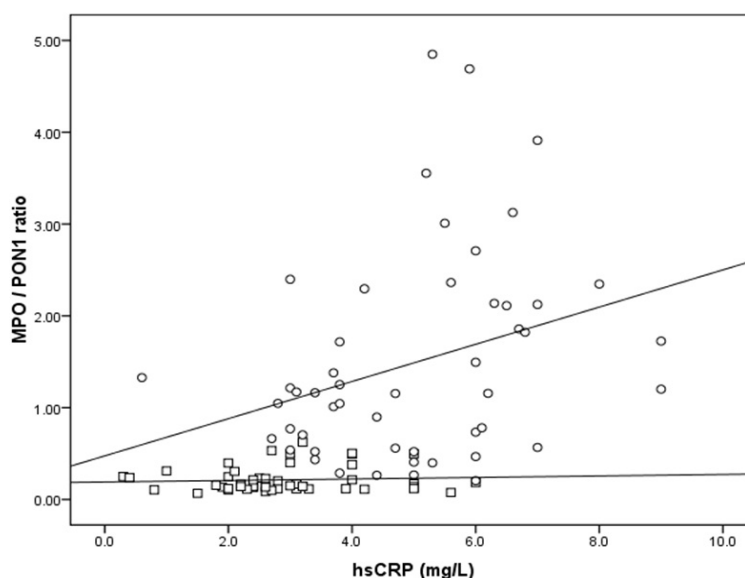


Figure 2. The correlation between MPO/PON1 ratio and hsCRP level in control subjects (□) and patients with ACS (○).

Table 2. Bivariate correlations between clinical biomarkers and MPO/PON1 in all subjects.

Variable	MPO/PON1	P value
Age (year)	0.27	< 0.01
BMI (kg/m ²)	0.28	0.01
Total cholesterol (mg/dl)	0.05	0.60
Triglycerides (mg/dl)	0.09	0.33
LDL-cholesterol (mg/dl)	0.15	0.15
HDL-cholesterol (mg/dl)	0.11	0.27
HDL mean size (nm)	0.31	0.03
hsCRP (mg/l)	0.52	< 0.01

BMI, body mass index; LDL-cholesterol, low density lipoprotein-cholesterol; HDL-cholesterol, high density lipoprotein-cholesterol; hsCRP, high sensitive C-reactive protein; PON1, paraoxonase-1; CAD, coronary artery disease.

The correlation between MPO/PON1 ratio and hsCRP is depicted in figure 2. As shown the correlation between MPO/PON1 ratio and hsCRP level was also significant only in patients with ACS ($r=0.31$, $P=0.003$).

The correlation between MPO levels and HDL particle size was only evidence in patients with ACS ($r=0.41$, $P=0.05$).

In a multiple regression analyses, adjusted for the other conventional covariates, MPO/PON1 was remained to be a significant predictor of ACS (OR=2.2, $P=0.01$). Interestingly, MPO was also a fair predictor of ACS (OR=1.8, $P=0.04$). Other clinical risk factors were not shown to be significant predictors of ACS.

Discussion

This study explicitly demonstrated that there is an obvious relationship between MPO/PON1 ratio and HDL mean size in patients with ACS. The significant correlations between MPO/PON1 and the risk factors, hsCRP, BMI, and HDL mean size but not lipid profile in the ACS patients, reflected a complicate interaction between MPO/PON1 and HDL function that may be, in part, through inflammatory mechanisms, given that ACS is an acute inflammatory condition. According to our results, there was no such significant correlation between PON1 and HDL particle size while the correlation between MPO/PON1 ratio and HDL particle size was inversely significant. This finding may introduce a possible causal relationship between MPO and HDL particle size in patients with ACS. Such a correlation was not found in control subjects. In accordance with our results there is a report by Karim *et al.* in which an independent association between HDL

particle size and cardiovascular risk factors was found (17). Also these researchers did not observe a strong relation between HDL particle size and PON1 levels. Recently, MPO/PON1 ratio has been suggested as a novel biomarker in secondary prevention of CAD (23). Moreover a correlation between MPO levels and unstable CAD as an ACS condition has also been reported (24). Accordingly, in the present study we found a significant association between MPO/PON1 ratio and ACS. The absence of a significant correlation between PON1 and HDL particle size may demonstrate that MPO has a higher impact on HDL function. This result is in good agreement with a recent study in patients with cardiovascular disease and type 2 diabetes mellitus (25). Also, in supporting of this notion a recent study in a large population in the Norfolk (UK), demonstrated that PON1 activity modestly predict the risk of future CAD and is not a causal factor in atherogenesis (26). According to some studies, the antioxidative capacity and therefore the atheroprotective effects of small and dense HDL particles are more than the large and light ones (14,15). The strong correlation between MPO or MPO/PON1 ratio and HDL particle size in patients with ACS in our study are all in good agreement with the mentioned studies. It has been proposed that phospholipids depletion may produce small HDL particle (27) and this may be the cause of oxidative resistance of the small HDL particles against oxidant agents such as MPO. The inverse correlation between MPO or MPO/PON1 ratio and HDL particle size in our patients are in good concordance with the notion.

The presence of the significant correlation between MPO/PON1 ratio and hsCRP in the ACS patients may further imply the involvement of inflammatory mechanism in the pathogenesis of ACS; however the deduction needs further evaluation. In conclusion, collectively, the present study demonstrated that MPO/PON1 ratio may be a robust predictor for the presence of ACS. Given the atheroprotective effects of small and dense HDL particles, the positive observed correlation between MPO/PON1 ratio and HDL mean particle size in ACS patients may reflect the implication of MPO/PON1 ratio in the pathogenesis of ACS through HDL dysfunction.

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