

Association between Adipokine and Myeloperoxidase Levels in Patients with Coronary Artery Disease

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Abstract- The adipokines, leptin and adiponectin, have a prominent role in the pathogenesis of coronary artery disease (CAD). The inflammatory enzyme, myeloperoxidase (MPO) also has an important role in the pathogenesis of CAD. Association of the adipokines with MPO remains to be resolved in patients with CAD. In this case-control study, 100 patients with CAD and 100 control subjects were appropriately recruited. Angiographic evaluation assigned the presence of CAD. Plasma leptin, adiponectin and MPO concentrations were measured using immunoassay methods. Other conventional cardiovascular risk factors were also recorded. Leptin and MPO concentrations were significantly increased in CAD patients compared to control subjects (25.38 ± 5.91 ng/ml vs. 3.68 ± 1.95 ng/mL and 52.85 ± 12.90 ng/mL vs. 23.00 ± 3.60 ng/mL, $P=0.001$, respectively). In contrast, adiponectin was significantly decreased in CAD patients compared to control subjects (5.62 ± 1.15 μ g/mL vs. 9.25 ± 1.8 , $P = 0.001$). There was a strong positive association between leptin and MPO concentrations only in CAD patients ($P = 0.01$). In contrast, a significant inverse association was found between adiponectin and MPO concentrations in CAD patients ($P = 0.01$). The associations also were significant after adjustment for other conventional risk factors ($P = 0.001$). Considering the presence of significant association between leptin and MPO, as well as adiponectin and MPO in patients with CAD, it may be inferred that the contribution of the adipokines in the pathogenesis of CAD may be, in part, through affecting the MPO concentration.

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Introduction

Adipose tissue secretes many pro- and antiatherogenic adipokines such as leptin and adiponectin that play an important role in the pathogenesis of atherosclerosis and coronary artery disease (CAD) (1). The adipokines, leptin and adiponectin, are considered as a potentially important link between systemic inflammatory processes and cardiovascular disease (2-3). Plasma adiponectin concentration has shown to be decreased in patients with CAD (4). Decreased serum adiponectin concentration is associated with the presence of cardiovascular disease and seems to be a robust predictor of CAD (5-7).

The involvement of leptin in the pathogenesis of

CAD is reported in many studies. In the prospective West of Scotland Coronary Prevention Study, it was shown that increased leptin concentration is moderately associated with increased risk of CAD (8). Leptin is elevated in patients with acute coronary syndrome and represent a significant association with other inflammatory markers of CAD (9). In another study, plasma leptin concentration is shown to be an independent predictor of cardiovascular events in patients with established coronary atherosclerosis (10). Overall, it is well established that the adipokines, leptin and adiponectin, contribute to the development of inflammatory diseases related to obesity (11).

Myeloperoxidase (MPO) is a leukocyte-derived pro-inflammatory and pro-atherogenic enzyme that

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participates in the processes of development, progression and complication of CAD through various mechanisms (12). MPO converts low-density lipoprotein (LDL) into an atherogenic form, oxidized LDL (Ox-LDL), provides oxidative stress condition and decreases nitric oxide (NO) bioavailability, all leading to atherosclerosis (13-16).

A few studies have explored the association of leptin and adiponectin with MPO. In one study, an inverse association was observed between low molecular weight adiponectin concentration and MPO in patients with type 2 diabetes mellitus (17). Moreover, a positive correlation was found between plasma leptin and MPO concentration in rats with intestinal inflammatory disease (18). Despite the aforementioned studies, the association between the adipokines and MPO in patients with CAD is yet to be determined. Therefore, we assessed a possible link between leptin, adiponectin and MPO in patients with CAD to clarify the contribution of the adipokines in the pathogenesis of CAD.

Materials and Methods

Participants

Patients were selected from subjects who had consecutively undergone diagnostic coronary angiographic procedure at the Isfahan Cardiovascular Research Institute, Isfahan, Iran. A total of 100 patients with CAD and 100 control subjects were enrolled in the study. Patients had an artery stenosis $\geq 50\%$ in at least one of the major coronary arteries. Patients with recent (within the past three months) myocardial infarction, chronic or acute inflammatory diseases, malignancies and surgical operations during the previous three months were not included in the study. Participants of the control group had not any history of cardiovascular diseases, and their coronary artery stenosis was less than 25%. This study was approved by the ethical committee of the Institute, and all participants provided written informed consent. The required information about traditional risk factors and medications were obtained via standardized questionnaire.

Biochemical measurements

An EDTA-plasma sample was prepared from every participant after a 12-hour fasting state and prior to the angiographic examination. The samples were aliquoted and stored at $-70\text{ }^{\circ}\text{C}$ until the measurement of biochemical variables. Plasma MPO concentration was quantified with a commercial immunoassay kit (Immunology Consultants Laboratory, USA). The intra-

assay and inter-assay coefficients of variations (CVs) for the measurement were 2.8% and 6.5%, respectively.

Plasma adiponectin and leptin concentrations were also quantified with enzyme-linked immunosorbent (ELISA) kits (Signosis, USA and ENZO Life Sciences, UK, respectively) according to the protocols of their manufacturers. The intra-assay and inter-assay CVs for the adiponectin assay were 3.2% and 6.5%, respectively. Also, the intra-assay and inter-assay CVs for the leptin determination were 4.2% and 6.7%, respectively.

The amount of plasma high sensitivity C-reactive protein (hs-CRP) was measured using a latex-enhanced immunoturbidimetric assay kit (Roche Diagnostics, Germany). Plasma lipid profile (total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol) was assayed by using standard enzymatic kits (Parsazmun, Karaj, Iran).

Statistical analysis

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL). The normally distributed data were reported as means \pm SD. Logarithmic conversion was performed on skewed variables. For comparing dichotomous variables, Chi-square test was used. Pearson's correlation coefficient analyses were applied to determine the correlation between variables with a normal distribution. A multiple logistic regression analysis was used to assign the independent predictors of plasma MPO concentration. A P -value < 0.05 was considered statistically significant.

Results

Clinical and biochemical characteristics of the patients and control participants are presented in Table 1. CAD patients and control group were age and gender matched. As showed in table 1, the cardiovascular risk factors were more prevalent in the patient group.

In patient's group, concentration of MPO and leptin, were significantly higher than in the control group. However the concentration of adiponectin was significantly lower in the patient group.

A significant association was found between leptin and MPO or adiponectin and MPO in patients with CAD (Figure 1).

As presented in the figure, the association between leptin and MPO was direct; however the correlation between adiponectin and MPO was inverse. Furthermore, we found a relatively significant association between leptin and hsCRP ($r=0.31$, $P=0.01$) as well as adiponectin and hsCRP ($r=-0.28$, $P=0.02$) in

CAD patients. In multiple logistic regression analysis, the association of adiponectin and leptin with MPO also was significant after adjustment for other conventional risk factors (Table 2). However, the association between

leptin and MPO or adiponectin and MPO was not statistically significant in the control group ($r=0.09$, $P=0.38$ for leptin; $r=0.19$, $P=0.06$ for adiponectin).

Table 1. Clinical and biochemical characteristics of participants

Variable	Control subjects (n =100)	CAD Patients (n =100)	P-value
Age (year)	57.24 ± 6.35	56.86 ± 8.56	0.61
Men	69 (69%)	70 (70%)	0.31
BMI (kg/m ²)	23.38 ± 1.81	26.49 ± 2.41	0.02
Cholesterol (mg/dL)	169.76 ± 37.10	170.94 ± 39.42	0.18
Triglyceride (mg/dL)	139.54 ± 98.43	141.29 ± 96.34	0.22
LDL (mg/dL)	111.77 ± 15.58	114.94 ± 32.38	0.12
HDL (mg/dL)	44.55 ± 5.45	42.14 ± 7.30	0.39
hs-CRP (mg/L)	1.41 (0.92-2.98)	2.86 (1.84-4.99)	0.008
MPO (ng/mL)	23.00 ± 3.60	52.85 ± 12.90	0.001
Leptin (ng/mL)	3.68 ± 1.95	25.38 ± 5.91	0.001
Adiponectin (µg/mL)	9.25 ± 1.80	5.62 ± 1.15	0.001
Hypertension	8 (8%)	25 (25%)	0.001
Diabetes mellitus	7 (7%)	23 (23%)	0.001
Family history of CAD	15 (15%)	35 (35%)	0.001
Smoking	12 (12%)	25 (25%)	0.001
Aspirin	0 (0%)	68 (68%)	0.0001

Data are presented as mean ± SD, medians (interquartile range) or number (%). BMI: body mass index, LDL: low-density lipoprotein, HDL: high-density lipoprotein, hs-CRP: high sensitive C-Reactive Protein, MPO: myeloperoxidase, CAD: coronary artery disease.

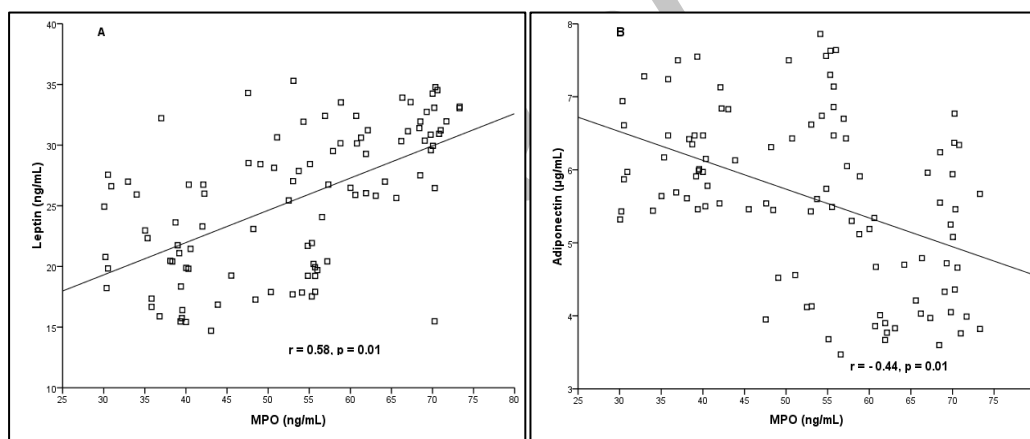


Figure 1. Association between leptin and myeloperoxidase (MPO) (A) or adiponectin and MPO (B) in patients with CAD. As showed, the association between leptin and MPO was direct; however the association between adiponectin and MPO was inverse.

Table 2. Association of independent variables with MPO concentration in patients with CAD

Variables*	OR	(95% CI)	P-Value
BMI (kg/m ²)	1.71	1.28-3.10	0.001
hs-CRP (mg/L)	1.63	1.35-2.77	0.001
Leptin (ng/mL)	2.39	1.98-3.76	0.001
Adiponectin(µg/mL)	0.43	0.23-0.51	0.001
Hypertension	1.49	1.12-2.35	0.001
Diabetes mellitus	1.74	1.53-3.82	0.001
Family history of CAD	1.47	1.23-2.76	0.03
Smoking	1.77	1.25-2.66	0.001
Aspirin	0.51	0.34-0.61	0.001

* Variables were different between patients and control subjects. OR: odds ratio, CI: confidence interval, BMI: body mass index, hs-CRP: high sensitive C-Reactive Protein, CAD: coronary artery disease.

Discussion

In the current study, to our knowledge, for the first time it was revealed that a significant association is present between the adipokines, leptin and adiponectin, and MPO in CAD patients. Leptin showed a positive, independent association with MPO. In contrast, adiponectin showed an inverse independent correlation with MPO.

Although the precise role of leptin in the cardiovascular system has not been determined, the relation of hyperlipidemia with poor prognosis of CAD is unequivocal (8,10). Leptin exerts many atherogenic effects; it promotes oxidative stress in endothelial cells, stimulates arterial smooth muscle cell migration and proliferation, decreases vascular expandability and provokes hypertension that all are collectively involved in the pathophysiology of atherosclerosis (19-20). Moreover, implication of MPO in CAD through various mechanisms, especially, the intensified inflammatory and oxidative stress conditions have been clearly determined (12). In the present study, higher concentrations of leptin and MPO and the observed independent correlation between them in CAD patients, raises the possibility that some atherogenic effects of leptin may be exerted through increasing MPO concentration. In this regard, the existence of a positive correlation between leptin and MPO in rats with intestinal inflammatory disease (18) may provide evidence regarding the association between leptin and MPO in inflammatory related conditions. Furthermore, many in-vitro and in-vivo studies have indicated that leptin promotes neutrophil and macrophage activation, enhances neutrophil proliferation (21, 22), and is associated with increased plasma hsCRP concentration (9). Accordingly, we observed a relatively significant positive association between leptin and hsCRP in patients with CAD. It should be considered that hsCRP stimulates MPO release from neutrophils and monocytes (23) and the MPO is predominately secreted by activated neutrophils and macrophages (12). Given the above evidence and also according to present findings, it would be conceivable to infer that leptin indirectly increases plasma MPO concentration, possibly in part through hsCRP effects and neutrophils and macrophages activation, although other mechanisms could not be ruled out.

Adiponectin, contrary to leptin, has been identified as an anti-atherogenic adipokine (24). In the present study, plasma adiponectin concentration was inversely

correlated with plasma MPO concentration. In accordance with current findings, an inverse correlation was reported between low molecular adiponectin and MPO in patients with type 2 diabetes mellitus (17), as well as between adiponectin and MPO in patients with acute myocardial infarction (25). Adiponectin has an inhibitory influence on proliferation of polymorphonuclear cells' (PMNs) lineage and on the function of matured macrophages (26). Therefore, the inverse association that we found between adiponectin and MPO in CAD patients may come from, in part, the inhibitory effects of adiponectin on PMNs and macrophages.

The results of multiple logistic regression analyses revealed that leptin and adiponectin, among other clinical risk factors, were the most important predictors of plasma MPO in CAD patients. Given the involvement of the adipokines, leptin and adiponectin (11), as well as MPO (12) in oxidative stress and inflammatory related conditions such as CAD, the relations would not be irrelevant. Overall, the associations found in this study between the adipokines, leptin and adiponectin, and MPO in CAD patients may be, in part, secondary to their effects on the PMNs and neutrophils.

In conclusion, according to the results of the present study, adiponectin inversely and leptin directly affect concentration of MPO in patients with CAD. Thus, several atheroprotective effects of adiponectin or atherogenic properties of leptin may be secondary to their effects on MPO concentration in patients with CAD.

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