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Plasma Levels of Inflammatory Biomarkers in Peripheral Arterial Disease: Results of a Cohort Study

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Abstract

Previous research analyzed the level of plasma inflammatory markers in patients with coronary disease, but very few studies have evaluated these markers in patients with peripheral arterial disease (PAD). The objective of this study was to investigate the plasma levels of inflammatory markers in patients with PAD and in healthy controls. The following plasma levels of biomarkers were measured in 80 patients with PAD (mean age 68 $\pm$ 5 years) and in 72 healthy participants (mean age 67 $\pm$ 6 years): interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), L-selectin (LS), neopterin (N), P-selectin (PS), E-selectin (ES), vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), and matrix metalloproteinase 2 (MMP-2), and 9 (MMP-9). Significantly higher levels of IL-6 ($P < .001$), TNF-α ($P < .0001$), ES ($P < .0001$), LS ($P < .0001$), PS ($P < .0001$), ICAM-1 ($P < .001$), VCAM-1 ($P < .001$), N ($P < .001$), MMP-2 ($P < .001$), and MMP-9 ($P < .005$) were found in the patients with PAD. Patients with PAD show a inflammation marker profile different from that of control participants. Reducing the high plasma levels of inflammatory markers could be a new therapeutic approach both for the prevention and the treatment of PAD.

Keywords

peripheral arterial disease, atherosclerosis, inflammation, biomarkers

Introduction

Peripheral arterial disease (PAD) is associated with a significant risk of cardiac and carotid events[1,2] and impaired blood flow in the lower limbs that causes intermittent claudication.[3,4] Atherosclerosis represents the principal pathogenetic reason for PAD, and it is a complex process that is influenced by many factors (eg, dyslipidemia, diabetes, hypertension, and smoking). Moreover, dysfunction of cells in the bloodstream (red and white blood cells and platelets) and endothelium play a significant role in the atherosclerotic process.[5,6] Proinflammatory molecules also contribute to atherogenesis.[7] Previous research[8-13] analyzed the level of plasma inflammatory markers in patients with coronary disease, but few studies assessed these markers in patients with PAD.[14]

The objective of this research is to establish whether the plasma levels of inflammation markers in patients with PAD were different from those in control participants. We considered interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), L-selectin (LS), and neopterin (N) as markers of immunological activation of leukocytes and macrophages.[15-17] P-selectin (PS) was considered as a marker of platelet activation.[18,19] E-selectin (ES),[20] intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule (VCAM-1) were used as markers of endothelial dysfunction.[21] Matrix metalloproteinase 2 (MMP-2) and 9 (MMP-9) were used as markers of extra cellular matrix remodeling.[22]

Methods

The present study included 80 patients with PAD and 72 patients without PAD as controls. Both patients and controls were recruited from patients admitted to the Medical Angiology Unit of Hospital Garibaldi (Catania, Italy). The mean age of the patients with PAD was 68 $\pm$ 5 years and that of controls

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Table 1. Characteristics of Patients With Peripheral Arterial Disease (PAD) and Controls Enrolled in the Study.

Variables	PAD (n = 80)	Control Participants (n = 72)	*P*
Age, years	68 ± 5	67 ± 6	ns
Dyslipidemia, Y/N	n = 51/29	n = 49/23	ns
Diabetes, Y/N	n = 55/25	n = 50/22	ns
Hypertension, Y/N	n = 41/39	n = 39/33	ns
Smoking, Y/N	n = 61/19	n = 58/14	ns
ABI	0.75 ± 0.12	1.2 ± 0.09	<.001

Abbreviations: Y, patients with risk factors; N, patients without risk factors; ABI, mean values of ankle/brachial index; ns, not significant.

was 67 ± 6 years. The PAD was diagnosed based on ankle–brachial index (ABI) values ≤0.9. The ABI was calculated by dividing the lower pressure measurement of the 2 posterior tibial arteries by the brachial artery pressure. The blood pressures in the lower limbs were measured with a Microdop 2 handheld continuous wave Doppler probe (SonoMed, France). The mean value of ABI in patients with PAD was 0.75 ± 0.12 and in the controls it was 1.2 ± 0.09. The characteristics of both PAD and normal participants are shown in Table 1. Individuals with acute myocardial infarction within the past year or who underwent coronary bypass surgery within the past 6 months were excluded from the study. Individuals with congestive heart failure, end-stage renal disease, chronic liver disease, autoimmune disease, immunodeficient conditions, or cancer were also excluded. Patients with PAD using vasoactive drugs and statins for >6 months were asked to discontinue these drugs for 2 weeks prior to measurement of inflammatory markers. We asked patients to stop both statin and other vasoactive drugs in order to measure all markers in baseline condition without the effect of the drugs.

Measurement of Markers in the Plasma

The concentrations of IL-6 and TNF-α were determined in the plasma according to the manufacturer's instructions (using commercial kits from Bender Med System, Wien Austrian) and an enzyme-linked immunosorbent assay (ELISA) reader (HR 700 Dynatech Lab). E-selectin, P selectin, L-selectin, ICAM-1, and VCAM-1 were determined in the plasma using commercial immunoassay kits (Bender Med System, Vienna, Austria). The sensitivity limits were L-selectin 0.3 ng/mL, E-selectin 0.5 ng/mL, P-selectin 1.3 ng/mL, soluble ICAM-1 0.5 mg/mL, and soluble VCAM-1 0.9 ng/mL. Both MMP-2 and MMP-9 were measured according to the manufacturer's instructions (Amersham Bioscience, United Kingdom) using an ELISA method; adsorbance of samples at 450 nm was measured by a spectrophotometer (ThermoLabsystems, Finland). Plasma levels of these markers were expressed in ng/mL. Neopterin (N) concentration was measured by an ELISA assay technique using commercially available reagents following the manufacturer's instructions (IBL Hamburg, Germany). The reference range of N was between 11 and 27 ng/L. All the blood samples were obtained from antecubital veins in the fasting state in the morning. The participants fasted for 12 hours, and no water intake was allowed. The samples were frozen at −20°C, and all the assays were performed in a single analysis.

Table 2. Mean Values of Biomarkers Measured in the Plasma of Patients With Peripheral Arterial Disease (PAD) and Normal Participants.[a]

Variables	PAD (n = 80)	Control Participants (n = 72)	*P*
IL-6, ng/dL	11.8 ± 1.2	7.3 ± 1.2	<.001
TNF-α, ng/dL	14.5 ± 3.3	9.3 ± 2.4	<.0001
ES, ng/dL	66.7 ± 1.2	56.6 ± 1.5	<.0001
LS, ng/dL	10.0 ± 3.2	5.5 ± 0.1	<.0001
PS, ng/dL	139 ± 2	107 ± 2	<.0001
ICAM-1, ng/dL	3.17 ± 0.4	2.08 ± 0.5	<.001
VCAM-1,ng/dL	4.85 ± 1.0	4.64 ± 1.10	<.001
MMP-2, ng/mL	1121 ± 456	701 ± 362	<.001
MMP-9, ng/mL	39 ± 24	25 ± 17	<.005
N, nmol/L	9.4 ± 4.6	5.3 ± 3.2	<.001

Abbreviations: IL-6, interleukin 6; TNF-α, tumor necrosis factor α; ES, E-selectin; LS, L-selectin; PS, P-selectin; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular adhesion molecule 1; MMP-2, metalloproteinase 2; MMP-9, metalloproteinase 9, N = neopterin.
[a]Values are expressed as mean ± standard deviation.

Statistical Analysis

The mean of the serum levels of inflammatory markers among individuals with PAD were compared to those of control participants using statistical techniques based on the Student *t* test and by the 1-way analysis of variance (ANOVA). A linear regression test was performed between N and ABI to investigate the relationship between inflammatory variables and PAD. Statistical analysis was performed using SPSS 10.1 software (SPSS Incorporated, United States). A 2-tailed $P < .05$ was considered significant.

Results

Table 2 shows that individuals with PAD had higher plasma levels of all inflammatory markers. The mean serum IL-6 concentration was 11.8 ± 1.2 ng/dL among individuals with PAD (controls 7.3 ± 1.2 ng/dL). The mean serum TNF-α concentration among individuals with PAD was 14.5 ± 3.3 ng/dL (controls 9.3 ± 2.4 ng/dL). Mean serum concentrations of ES, LS, and PS among individuals with PAD were 66.7 ± 1.2, 10.0 ± 3.2 and 139 ± 2 ng/dL, respectively (controls 56.6 ± 1.5, 5.5 ± 0.1, and 107 ± 2 ng/dL, respectively). Mean serum concentrations of ICAM-1 and VCAM-1 among individuals with PAD were 3.17 ± 0.4 and 4.85 ± 1.0 ng/dL, respectively (controls 2.08 ± 0.5 and 4.64 ± 1.10 ng/dL). The mean serum N concentration was 9.4 ± 4.6 nmol/L in patients with PAD (controls 5.3 ± 3.2 nmol/L). Mean serum concentrations of MMP-2 and MMP-9 among individuals with PAD were 1121 ± 456 and 39 ± 24 ng/mL, respectively (controls 701 ± 362 and 25 ± 17 ng/mL, respectively).

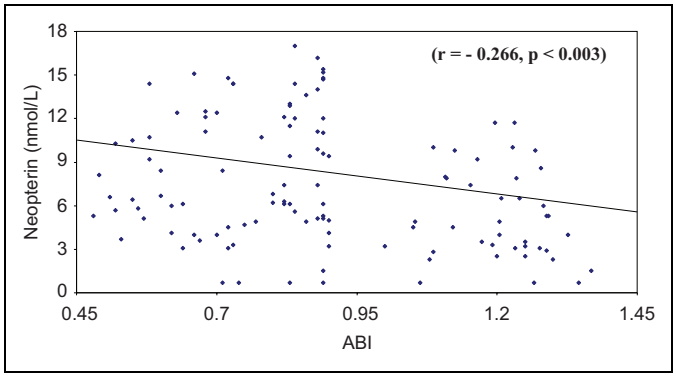

Figure 1. Inverse correlation between ankle–brachial index (ABI) values and neopterin (N) concentration.

We performed a regression analysis between N and ABI to elucidate any direct relationship between inflammation and the clinical condition. We considered these 2 variables because N represents a specific marker of activated macrophages,[15-16] and the ABI is a specific marker for PAD.[23-26] An inverse correlation ($r = -.266$, $P < .003$) was found in patients with PAD (Figure 1)

Discussion

Inflammation is an important contributor to atherosclerosis,[5-7] and high levels of inflammatory markers are associated with atherosclerotic disease. Blood-borne inflammatory mediators cause damage to the arterial endothelium which, in turn, induces proliferation of smooth muscle cells. Therefore, plasma levels of inflammatory mediators have been used as markers of arterial wall damage.[27]

Previous research established the importance of inflammation in coronary artery disease.[28] Patients with PAD also show increased plasma levels of inflammatory markers, including C-reactive protein, fibrinogen, amyloid A, MMP-2, and MMP-9 compared to controls.[29-31] It is known that patients with PAD have an increased morbidity and mortality from cardiovascular disease, and this is associated with the presence of elevated circulating levels of inflammatory markers.[14,32-33] However, it is not clear whether these markers are of clinical relevance in patients with PAD. It is of interest that antiplatelet drugs (eg, acetylsalicylic acid and clopidogrel) can reduce both the levels of CRP or transcription factor nuclear factor kappa B and/or reduce the serum levels of CD40 ligand and PS.[34] Furthermore, data from clinical studies suggest that antiplatelet therapy may attenuate the release of inflammatory mediators.[34] Our results are in agreement with previous findings[27-30] in demonstrating that plasma levels of several mediators of inflammation (eg, IL-6, TNFα, ICAM-1, VCAM-1, ES, LS, PS, and N) are increased in patients with PAD compared to controls. Interleukin 6 can contribute to the atherosclerotic process by upregulating fibrinogen and acute-phase reactant production by enhancing adhesiveness of endothelial cells and by activating production of tissue factor and von Willebrand factor.[14] Moreover, the adhesion molecules play a role in leukocyte adhesion in the vessel wall and also in the migration of these cells into the arterial wall; both these events are crucial in the atherosclerotic process.[35] We previously found[36] that levels of selectins were higher in patients with PAD than in controls at rest, and these serum levels increased after a treadmill test both in PAD and control patients. Raised serum levels of the selectins were higher and more significant in patients with PAD than in controls.

Based on these results, we postulated that hypoxia related to increased muscular effort causes both activation of white blood cells and also the release of inflammatory mediators in the blood stream. Our results are consistent with the hypothesis that inflammation plays a key role in the pathogenesis of PAD. We would also like to underline the difference found among MMPs 2 and 9 between patients with PAD and normal participants. These gelatinases interact with the matrix of the arterial wall,[37] and they are released by numerous proteinases (ie, neutrophil elastases). Furthermore, they are released in the extracellular compartment through oxidative substances (ie, oxidized glutathione).[22] These proteinases also play a role in arterial wall remodeling.[38]

Higher levels of MMPs, especially of MMP-9, were found in aneurysmal aortas and in atherosclerotic femoral and carotid arteries. Close relationships were found between higher levels of MMP-9 and carotid atherosclerotic plaque, and a relationship was found between high serum levels of MMPs and high risk of atherosclerotic disease.[30,39-42] Based on these results, the level of MMPs was considered as a marker for inflammation and also an index of plaque activity. Furthermore, using these different markers, we can assess the remodeling activity of the arterial wall that is influenced by inflammation.

Our findings suggest that treatments aimed at reducing inflammation may be effective in preventing the progression of atherosclerosis and improving clinical outcome in patients with PAD. Further studies in patients with PAD will need to determine whether anti-inflammatory therapies are effective for the prevention and treatment of PAD and its associated cardiovascular complications. Possible limitations of this study could be its observational nature. Hypolipemic drugs can lower the level of inflammatory markers.[43,44] Other research will need to demonstrate that medical strategies to minimize the release of inflammatory products could be efficacious also in treating patients with PAD.

Authors' Note

All authors contributed to (1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published.

Declaration of Conflicting Interests

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