Correlation between the levels of circulating adhesion molecules and atherosclerosis in type-2 diabetic normotensive patients

Circulating adhesion molecules and atherosclerosis

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Endothelial dysfunction is a common feature in type-2 diabetic patients and is associated with inflammation, increased levels of circulating soluble adhesion molecules and atherosclerosis. The aim of this study was to evaluate the relationship between the levels of circulating soluble adhesion molecules and the degree of atherosclerosis in normotensive type-2 diabetic patients.

Results: We found significant correlations between ICAM-1 (r = 0.69, p < 0.001 95% IC 0.65 to 0.82) and VCAM-1 (r = 0.4, p < 0.03, 95% IC 0.65 to 0.82) levels and maximal carotid artery intimal-medial thickness, whereas no correlation was observed with E-selectin.

Methods: We studied 30 normotensive type-2 diabetic patients in whom VCAM-1, ICAM-1 and E-selectin were measured by ELISA. Additionally, the intimal-medial thickness of both the common and internal carotid arteries was measured (B-mode ultrasound). The levels of circulating adhesion molecules and maximal carotid artery intimal-medial thicknesses were correlated using the Spearman correlation coefficient test. Statistical analysis was performed with ANOVA.

Conclusion: Our results suggest that ICAM-1 and VCAM-1 are markers associated, and correlated with the degree of atherosclerosis in normotensive type-2 diabetic patients.

Type-2 diabetic patients suffer from some form of endothelial dysfunction. Endothelial dysfunction in these patients stimulates inflammation and increases levels of circulating soluble adhesion molecules.¹ Both endothelial dysfunction and inflammation contribute to atherosclerosis via several mechanisms.²

Leukocytes are unable to adhere to normally functioning arterial endothelium; however, in the setting of endothelial dysfunction, the bioavailability of nitric oxide is reduced, resulting in the activation of nuclear factor κB (NFκB). NFκB increases proinflammatory gene expression, including the expression of leukocyte adhesion molecules,³ which are expressed on the arterial endothelium.⁴ Also, NFκB increases systemic concentrations of soluble forms of adhesion molecules (SAM), perhaps as a result of a proteolytic processing on the endothelial cell surface.⁴,⁵ Circulating levels of SAM are thought to reflect increased endothelial cell surface expression⁵ and high serum levels of SAM are considered markers of endothelial dysfunction.¹ Endothelial dysfunction seems to be the trigger in atherogenesis and diabetes-associated vascular disease and explains, at least in part, the enhanced progression of CVD (cardiovascular disease) in type 2 diabetics.

Several inflammatory markers, such as C reactive protein and interleukin 6, have been implicated in diabetic macrovascular complications.²,⁴ Early atherosclerosis has an inflammatory component characterised by leucocytic infiltration of the vascular endothelial wall.⁶ SAMs may be implicated in the development of the atherosclerotic plaque by facilitating the attachment and migration of leukocytes into the arterial wall, which is a critical early step in the initiation of atherosclerosis.⁵,⁷ In fact, genetically modified mice that lack expression of adhesion molecules do not develop atherosclerosis and the administration to mice of antibodies directed against SAM decreases intimal hyperplasia.⁶ Adhesion molecules have been observed consistently within the milieu of the atherosclerotic plaque,²,⁷ but it is not clear whether circulating levels of SAM are associated with atherosclerosis, specifically in type-2 diabetic patients, in whom circulating levels of SAM are increased.⁸,⁹

High-resolution B-mode ultrasonography provides a noninvasive method of quantifying subclinical arterial wall thickening and atherosclerotic progression. Ultrasound is preferable to arteriography because it is noninvasive, carries no risk for the examined subject, and can detect atherosclerosis as an increase in arterial wall thickness before a reduction in lumen diameter occurs. Increases
in the thickness of the intimal-medial layer are measured by ultrasonography and have been directly associated with an increased risk of myocardial infarction and stroke. The aim of this study was to evaluate whether relationships between circulating soluble adhesion molecules levels and the degree of atherosclerosis (defined as the maximal intimal-medial thickness) exist in normotensive type-2 diabetic patients.

Results

Baseline patient characteristics are depicted in Table 1. Circulating levels of SAM were: VCAM-1, 824.3 ± 99 ng/ml; ICAM-1, 277.6 ± 46 ng/ml; and E-selectin, 68.4 ± 13 ng/ml.

The intimal-medial thickness of the carotid arteries in our patients was 13.43 ± 3.4 μm.

We were unable to demonstrate any relationship between maximal intimal-medial thickness and E-selectin (r = -0.02, p > 0.1). However, we observed significant positive correlations (r = 0.4, p < 0.03, 95% IC 0.65 to 0.82) with VCAM-1 and ICAM-1 (r = 0.69, p < 0.001 95% IC 0.65 to 0.82) and the maximal intimal-medial thickness (Fig. 1).

Discussion

In this study, circulating levels of ICAM-1 and VCAM-1 were significantly correlated with the maximal intimal-medial thickness in normotensive type-2 diabetic patients, whereas no significant association was observed for E-selectin. Circulating levels of SAM were measured in duplicate, and, as a result, intra-individual variation was accounted for and born out in the statistical analysis. Interestingly, our patients were thiazolidinedione-naïve, statin-naïve and ACE/ARB-naïve. All of these drugs have been shown to reduce circulating levels of SAM.

It has been suggested that adhesion molecule levels may have a prognostic value in ischemic heart disease, and this issue remains controversial. Alterations in the vascular endothelium linked to diabetes that contribute to endothelial dysfunction include elevated plasma levels of adhesion molecules and associated enhanced adhesion of monocytes to vascular endothelium, plus impairment of NO release and reduced NO responsiveness. The adhesion and migration of circulating macrophages are important in the initiation and progression of atherosclerotic disease. These processes are mediated largely by cellular-adhesion molecules. Then, SAM must have a role in the progression of atherosclerosis. In fact, in the Edinburgh study, Tsoulaki et al. observed a correlation between ICAM-1 and peripheral atherosclerosis; however, only 6% of the patients were diabetic, and peripheral atherosclerosis was evaluated using the ankle-brachial index. In the Atherosclerosis Risk in Communities (ARIC) study, a correlation between ICAM and intimal-medial thickness was found. This study group demonstrated that E-selectin correlated with carotid changes, however, only 10% of the subjects were diabetic. Finally, Rizzoni et al. found an association between ICAM-1 and carotid artery structure changes in hypertensive type-2 diabetic patients, but not in normotensive diabetic patients.
In type 2 diabetes, increased vascular inflammation, including enhanced expression of cellular adhesion molecules are observed. Our results agree with the results of the papers above mentioned. However, those studies included few patients with type-2 diabetes; our study is the first one that found a correlation between circulating SAM levels and intimal-medial thickness in normotensive type-2 diabetic patients.

Both ICAM-1 and VCAM-1 have been implicated in leukocyte attachment and intimal penetration. The association between circulating SAM levels and intimal-medial thickness can be explained by a variety of observations. Excessive VCAM-1 expression has been reported in human smooth muscle cells and in endothelial cells overlying incipient atheroma. It has been hypothesized that intimal neovascularization may be an important site of white cell recruitment and inflammation, as seen in atheromatous changes. VCAM-1 binds the monocytes found in early human and experimental atheroma, and endothelial cells express VCAM-1 selectively in areas prone to lesion formation in response to cholesterol feeding. Alternatively, the production of ICAM-1 is increased in sites of disturbed flow, which are sites of lesion formation. These observations may contribute to the correlation between SAM levels and intimal-medial thickness; however, these issues require experimental corroboration.

Our results support the relationship between type-2 diabetes mellitus, endothelial dysfunction, inflammation and atherosclerosis. Additionally, they support, at least in part, the use of drugs such as statins and renin-angiotensin system inhibitors, both with anti-inflammatory effects, in the prevention of cardiovascular complications in normotensive type-2 diabetic patients. In conclusion, our data suggest that systemic levels of ICAM-1 and VCAM-1 are associated with intimal-medial thickness and correlated with the degree of atherosclerosis in type-2 diabetic normotensive patients.

**Materials and Methods**

A total of 30 normotensive patients with type-2 diabetes mellitus (>12 months), who were thiazolidinedione-naive, statin-naive and ACE/ARB-naive, were included in this study. The diagnosis of type-2 diabetes was performed according to the American Diabetes Association criteria. In all of them, VCAM-1, ICAM-1 and E-selectin circulating levels were measured in duplicate by commercial ELISA kits (R&D Systems, Minneapolis, MN). All venous samples were collected in the morning after a 12-h overnight fast. Intra-assay precision (precision within an assay) was 3.1% for VCAM-1, 4.1% for ICAM-1, and 3.8% for E-selectin, whereas inter-assay precision (precision between assays) was 7% for VCAM-1, 7.3% for E-selectin and 7.4% for ICAM-1. Also, fasting glycaemia (glucose oxidase) and HbA1c were measured from those samples.

Patients with any of the following diagnoses were excluded from the study:

- Decompensated diabetes mellitus (fasting blood glucose >250 mg/dl), heart, hepatic or renal failure, evidence of valvular heart disease; heart block or cardiac arrhythmia, hypertension (according to JNC 7 criteria), acute coronary syndrome or cerebrovascular disease six months before the study’s initiation; autoimmune disease, pregnancy; urinary tract infection, fever or a history of alcohol abuse and/or psychotropic drugs.

B-mode color imaging of extracranial carotid arteries was obtained using high-resolution ultrasound (ESAUTE MEGAGP, Italia) equipped with a 10 MHz linear transducer. Subjects were evaluated lying in the supine position with hyperextension of the neck. Measurements of the distal wall of both the common and internal carotid arteries were obtained. Registers were performed at the end of the diastole and all determinations were performed by the same certified ultrasonographer, who was blinded to the study.

**Statistical analysis.** Data are presented as the mean ± standard deviation. The relationship between circulating SAM levels and the maximal intimal-medial thickness was assessed by the Spearman rank correlation coefficient test.

The study was conducted with the approval of the Research and Medical Ethics Committee of our hospital, in accordance with the Helsinki declaration. Participants provided informed, written consent before their inclusion in the study protocol.

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