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BRACHIAL ARTERY VASODILATOR FUNCTION AND ADHESION MOLECULES IN RHEUMATOID ARTHRITIS

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ABSTRACT

Objective Cardiovascular (CV) event rates are increased in Rheumatoid Arthritis (RA). Traditional cardiovascular risk factors do not adequately account for the extent of cardiovascular disease in RA. The relation among biomarkers of endothelial dysfunction, inflammation and vascular function remain uncertain. Hence we investigated the relationship between biomarkers of endothelial dysfunction, inflammation and vascular function in RA patients.

Methods 30 adult RA patients (4 male, 26 female) and 30 age and sex matched healthy controls (6 male, 24 female) were enrolled in the study. Assessment of FMD done by Angiodefender™ (Everest Genomic, Ann Arbor, United States). Soluble adhesion molecules (sICAM-1 and sVCAM-1) and pro-inflammatory cytokines (TNF-α, IL-6 and IL-1) was measured using standard ELISA kits. As surrogates for disease activity, DAS28, C-reactive protein and ESR levels were determined.

Results Compared with healthy controls, RA patients had significantly ($p<0.05$) increased basal concentrations of soluble intracellular adhesion molecules (sICAM-1), soluble vascular cell adhesion molecule (sVCAM-1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1 (IL-1). Concentrations of sVCAM-1 related to TNF-α and FMD ($P<0.05$) while sICAM-1 related to IL-1 ($P<0.05$). sVCAM-1 related to DAS28 and CRP revealed a significant ($p<0.05$) positive correlation in RA patients

Conclusion In RA, endothelial activation correlates with FMD, through inappropriate secretion of cytokines. Our observations are consistent with the hypothesis that up regulation of adhesion molecules by inflammatory cytokines impairs vascular function. This suggests that strategies to decrease inflammatory activity in RA patients should focus not only on the treatment of conventional risk factors, but also on the improvement of endothelial function.

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INTRODUCTION

Cardiovascular (CV) disease continues to be the leading cause of morbidity and mortality in Rheumatoid Arthritis (RA) *[1]*. This is a

consequence of atherosclerosis *[2, 3]*. Atherosclerosis is now considered an inflammatory disease *[4]*. In RA, subclinical atherosclerosis, manifested by increased carotid artery intima-media thickness and increased number of carotid plaques, has been observed without classic CV risk factor or CV events *[5]*. In the process of accelerated atherosclerosis in RA, a major issue is the development of endothelial activation, which leads to endothelial dysfunction and premature atherosclerosis. Increased levels of circulating inflammatory mediators are believed to cause activation and damage of endothelial cells (EC) in patients with RA *[4]*.

Endothelial status may be regarded as an integrated index of all atherogenic and atheroprotective factors present in an individual *[6]*. Endothelial dysfunction is an essential initial step in atherogenesis and is a barometer of cardiovascular health *[7]*. A healthy endothelium prevents adhesion of mononuclear cells. Inflammation promotes the activation of endothelial cell, which is characterized by loss of vascular integrity, increased expression of adhesion molecules, such as selectins, VCAM-1 and ICAM-1, change in phenotype from antithrombotic to thrombotic and production of several cytokines. All these changes allow EC to participate in the inflammatory response. In this process, increased expression of adhesion molecules promotes the adherence and migration of monocytes into the vessel wall. Differentiation of monocytes into macrophages in the intima, activation and further differentiation to foam characterize the development of early atherosclerotic lesion *[8, 9]*. Elevated adhesion molecules are associated with cardiovascular risk factors *[10]* and predict atherosclerosis and cardiovascular events *[11-13]*. It has been reported that such biomarkers play a more important role than traditional risk factors in cardiovascular disease in RA *[14, 15]*. Important in this context is that high adhesion molecule levels may not only reflect synovial inflammation but also indicate exposure of the systemic vascular endothelium to high circulating cytokine concentrations *[16]*. To our knowledge, the relationship among biomarkers of endothelial dysfunction, inflammatory cytokines and vascular function has not been reported in RA. We decided to assess the association between biomarkers of endothelial dysfunction, inflammatory cytokines and vascular function in RA.

Material and methods

Patients

Thirty adult RA patients (4 male, 26 female; mean age 41.62 ± 1.72 years, range 21-55) who fulfilled the 2010 Rheumatoid Arthritis Classification Criteria for diagnosis and classification of RA *[17]* with active disease despite treatment with conventional DMARDs were enrolled in the study from a rheumatology outpatient clinic. All the patients included had active RA, defined by the presence of modified Disease Activity Score (DAS 28 > 3.2). Exclusion criteria included patients with diabetes mellitus, a past medical history of coronary artery disease, smokers, renal or hepatic

dysfunction. Concomitant use of drugs known to affect endothelial function (nitrates, statins, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and beta blockers). Thirty healthy subjects matched for age (mean age 40.8±1.3, range 24-56) and sex (M/F=6/24) acted as control.

The study protocol was approved by the regional ethical research committee and was performed in accordance with the declaration of Helsinki and the code of Good Clinical Practice. All patients provided written informed consent to participate after a full explanation of the study.

Assessment

In all subjects, blood was drawn in the morning after overnight fasting and the following variables were determined: complete blood count, erythrocyte sedimentation rate (ESR), C-reactive protein, renal and liver function tests, serum electrolytes and fasting blood sugar by conventional methods using standard commercial kits.

Assessment of Endothelial Function by Angiodefender

The AngioDefender™ (Everist Genomics, Ann Arbor, United States) procedure is non-invasive and employs neither ultrasound nor Doppler flow analysis. The AngioDefender device uses a novel, proprietary software algorithm to analyze pulse wave data collected before and after Brachial Artery (BA) occlusion by an upper arm sphygmomanometric cuff. At the end of the AngioDefender testing procedure (~15 minutes), the maximal relative post-occlusion change in the diameter of the BA relative to baseline is calculated and expressed as a percentage of flow mediated dilation (%FMD). AngioDefender testing is applicable to any patient, regardless of age, gender, ethnicity or pre-existing conditions. AngioDefender test results are not dependent on user technique or operator proficiency.

Assessment of inflammatory disease activity: The following measures of clinical evaluation of inflammation were employed.

Estimation of Pro-inflammatory Cytokines i.e. TNF-α, IL-6 and IL-1 was done by using standard ELISA kits (Diaclone SAS, France). Disease Activity Score of 28 joints (DAS28) was used to assess disease activity of 28 joints by a composite measure, which is a linear sum of four parameters including tender joint count (TJC), swollen joint count (SJC), patient global assessment of general health on a visual analogue scale (VAS). Erthrocyte sedimentation rate (ESR) was measured by Westergren method and C-reactive protein (CRP) level was determined using standard commercial kits.

Estimation of soluble Adhesion molecules:

Estimation of soluble Adhesion molecules i.e. sICAM-1 and sVCAM-1 was done by using standard ELISA kits (Diaclone SAS, France).

Statistical Analysis

Test values are reported as mean ± SEM. Spearman analysis was used to find the relationship between FMD, adhesion molecules and pro-inflammatory cytokines. A p value<0.05 was considered to indicate significant difference. Statistical analysis was

done using the Prism Software 5.04 version for Windows 8.0.

Results:

***Patient profile*:** The baseline demographic and clinical characteristics of the patients and controls are presented in Table 1.

Endothelial Function

FMD in RA patients was significantly impaired as compared with the healthy age and sex matched control group (5.5±1.4% vs. 10.7±2.1%, p<0.001) (Table 1).

Inflammatory Cytokines:

Levels of pro-inflammatory cytokines i.e. TNF-α (p=0.01), IL-6 (p=0.01), and IL-1 (p=0.01) were significantly higher in RA patients as compared healthy controls (Table 1).

***Inflammatory disease activity*:**

All patients included in the study had high disease activity (DAS28≥5.1). Levels of inflammatory disease activity measures i.e. DAS28 (p=0.01), ESR (p=0.01), CRP (p=0.03), IL-6 (p=0.01) were significantly higher in RA patients as compared with healthy controls (Table 1).

Adhesion molecules:

Levels of adhesion molecules i.e.sICAM-1 (p=0.01) and sVCAM-1(p=0.01) were significantly higher in RA patients as compared to healthy controls (Table 1) suggesting that higher levels of adhesion molecules are associated with impaired endothelial dysfunction in RA patients.

Association of adhesion molecule with inflammatory cytokines and endothelial dysfunction:

At Univariate analysis, sVCAM-1 was related to TNF-α (p=0.01) Fig.1) and FMD (p=0.01) (Fig.2) and sICAM-1 related to IL-1 (p=0.01) in (Fig.3) RA. Univariate regression was applied to determine whether an association existed between adhesion molecules and the inflammatory disease activity measures (DAS-28, ESR and CRP) in RA patients. Of the markers analyzed, sVCAM-1 related to DAS28 and CRP demonstrating a significant (p=0.01) positive correlation in RA patients (Table 2).

Discussion:

This study demonstrates for the first time that the relationship between brachial artery flow mediated vasodilation, inflammatory cytokines and adhesion molecules, previously demonstrated in Framingham Offspring study *[18],* also occur in patients with RA. This suggests that the expression of cell adhesion molecules is up regulated by pro-inflammatory cytokines and association with FMD responsible for endothelial dysfunction may have a central role in the mechanism of immune-mediated inflammation and atherosclerosis.

We found that sICAM-1 and sVCAM-1 were markedly higher in RA patients than in healthy controls. Previous reports have also shown elevated levels of serum soluble intracellular adhesion molecule-1 (sICAM-1), sICAM-3 and sP-selectin in RA *[19].* However, studies relating to inflammation and endothelium-dependent dilation have yielded conflicting results in the past. For example, CRP did not correlate with brachial artery flow-mediated dilation in patients with familial hypercholesterolemia, *[20]* Conflicting results have also been reported with regard to

the relation between endothelial function and sICAM-1 *[21, 22]*. The present study may provide a basis for reconciling this conflicting literature. Our, study findings support the possibility that systemic inflammation may represent a mechanistic link between adhesion molecules and vascular dysfunction.

TABLE 1. The demographic and clinical characteristics of the patients and healthy controls in Rheumatoid Arthritis.

	RA (n=30)	Control (n=30)	p-value
Patient characteristics			
Sex (F:M)	26:4	24:6	0.34
Age (years)	41.62 ± 1.72 (Range:21-55)	40.80±1.33 (Range:24-56)	
Disease duration (years)	6.63±1.02		
Height (cm)	159.4±4.9	160.7±4.95	0.21
Body weight (Kg)	64.07±12.58	63.13±12.51	0.43
BMI (kg/m^2)	24.43 ±5.2	24.13±2.91	0.37
ESR (mm 1st hr)	40.30±1.7	18.55±1.43	0.01*
CRP (mg/dl)	14.35±3.6	3.31±0.52	0.03*
DAS 28 Score	5.10±0.11		
TNF-α (pg/ml)	6.09±0.15	2.26±0.08	0.01*
IL-6 (pg/ml)	16.25±0.21	9.79±0.22	0.01*
IL-1 (pg/ml)	168.7±1.6	103.5±2.09	0.01*
FMD (%)	5.5±1.4	10.7±2.1	0.01*
VCAM-1 (ng/ml)	812±22.6	487±45.1	0.01*
ICAM-1(ng/ml)	221±1.1	121±1.2	0.01*

F female, M male, BMI body mass index, ESR erythrocyte sedimentation rate, CRP C-reactive protein, DAS 28- disease activity score of 28 joints, TNF-α Tumour Necrosis Factor, IL-6 Interleukin-6, IL-1 Interleukin-1, FMD Flow mediated vasodilation, ICAM-1 Intracellular Adhesion Molecule, VCAM-1 Vascular Adhesion Molecule. NS non significant, *p<0.05, Statistically significant

TABLE 2 Univariate correlations of Adhesion molecules with inflammatory markers and flow mediated vasodilation in Rheumatoid Arthritis

	sICAM-1		sVCAM-1	
Variables	**r**	**p-value**	**r**	**p-value**
Disease Duration	0.30	0.18	0.26	0.33
Disease Severity (DAS 28)	0.41	0.02*	0.45	0.02*
ESR	0.38	0.17	0.16	0.49
CRP	0.11	0.96	0.49	0.01*
IL-6	0.24	0.12	0.02	0.92
IL-1	0.48	0.02*	0.19	0.40
TNF-α	0.40	0.09	0.41	0.02*
FMD	0.39	0.21	-0.36	0.04*

DAS 28- disease activity score of 28 joints, ESR erythrocyte sedimentation rate, CRP C-reactive protein, IL-6 Interleukin-6, IL-1 Interleukin-l, TNF-α Tumour Necrosis Factor, FMD Flow mediated vasodilation, ICAM-1 Intracellular Adhesion Molecule, VCAM-1 Vascular Adhesion Molecule. *p<0.05, Statistically significant

We found a significant correlation between sVCAM-1 and vascular function which was estimated by FMD in RA patients which suggests that adhesion molecules may be the driving force for vascular dysfunction. This is the first study which shows a relationship between adhesion molecule and vascular function assessed by brachial artery flow mediated vasodilation. Previously the association of adhesion molecule i.e. sICAM-

1with vascular function has been shown in Framingham offspring study *[18].* This also opens the possibility of employing therapeutic agents to protect the vessels from the effects of adhesion molecules.

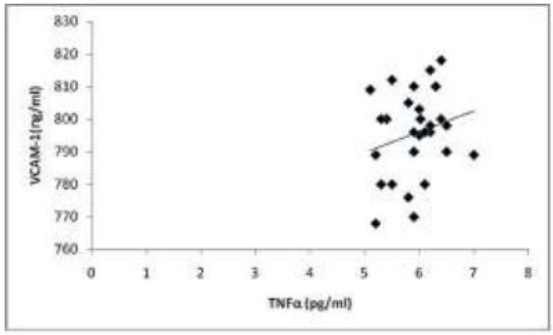

Fig.1 Correlation of Vascular Cell Adhesion Molecule-1 (VCAM-1) with Tumour Necrosis Factor-α (TNF- α) in RA.*p <0.05. Statistically significant.

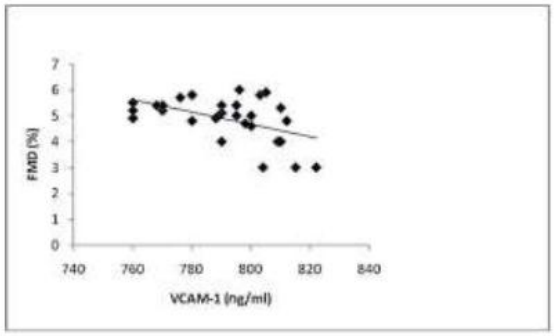

Fig.2 Correlation of Vascular Cell Adhesion Molecule-1 (VCAM-1) with Flow Mediated Dilation (FMD) in RA.*p <0.05. Statistically significant.

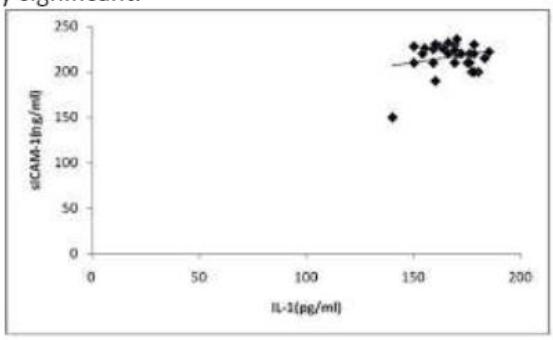

Fig.3 Correlation of Intracellular Adhesion Molecule-1 (ICAM-1) with Interleukin-1 (IL-1) in RA.*p <0.05. Statistically significant.

Inflammation is thought to play an important role in atherothrombosis *[8].* Inflammatory activity is increased in RA *[23],* a disease that confers a high risk of atherothrombosis. It is therefore important to investigate the determinants of inflammatory activity. The present study shows that, in RA, inflammatory activity assessed by proinflammatory cytokines was strongly associated with the adhesion molecules i.e. soluble VCAM-1 and soluble ICAM-1. We found significant correlation between sVCAM-1 and TNF-α and between sICAM-1 and IL-1. This suggests that increased concentration of inflammatory cytokines in RA is responsible for activation of these adhesion molecules

which are the biomarkers of endothelial dysfunction. Previous work in RA has shown association of adhesion molecules with cytokine i.e. IL-6 *[24]* but not with the IL-1 and TNF-α as shown in our study. It would appear from these observations that anti-cytokine therapy with biologic DMARDs should favourably impact the adhesion molecules and the associated vascular dysfunction.

sVCAM-1 has shown a significant correlation with DAS28 in the present study. Previous work in RA has shown weak correlation of ICAM-1 with joint score and ESR *[25]* but in our study a strong and significant correlation of sVCAM-1 with DAS 28 has found suggesting that inflammatory disease activity is responsible for activation of adhesion molecules in RA patients. A growing body of evidence implicates that CRP directly promote the up regulation of endothelial cell adhesion molecules such as ICAM-1, VCAM-1 & E-selectin and act as a mediator of endothelial dysfunction *[26, 27]*. In the present study, correlation of sVCAM-1 with CRP would support the hypothesis that CRP is responsible for upregulation of these adhesion molecules and the later play a key role in facilitating the leukocyte-endotelial interaction, an early step in atherogenesis.

In summary, information about the biomarkers of endothelial dysfunction can be of clinical relevance in RA. Endothelial dysfunction is well established therapeutic target in RA. Soluble VCAM-1 and soluble ICAM-1 may be new therapeutic targets. Several substances, such as statins *[28],* ACE inhibitors *[29],* and anti-oxidants *[30]* have been reported to reduce soluble E-selectin levels. We have shown that endothelial adhesion molecules are important determinants of vascular inflammation in RA patients. These data suggest that management strategies in RA patients should focus not only on the treatment of inflammatory disease activity and conventional risk factors, but also on the improvement of endothelial function. Our findings may have clinical relevance, because endothelial dysfunction is strongly linked to the pathogenesis and clinical expression of atherosclerosis. Longitudinal studies in relation to this may provide additional information about the links between systemic inflammation, vascular dysfunction and cardiovascular disease.

Key messages:

First study which shows a relationship between adhesion molecule and vascular function in RA.

This study opens the possibility of employing therapeutic agents to protect the vessels from the effects of adhesion molecules.

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Conflict of interest None.

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