

Correlation of Tissue Selectin Expression and Hemodynamic Parameters in Rheumatic Mitral Valve Disease

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Background and aim of the study: The study aim was to examine tissue expression of the adhesion molecules E-selectin and P-selectin on atrial, valvular and atrial myocardial blood vessel endothelium in patients with rheumatic mitral stenosis, and to investigate whether such expression was correlated with hemodynamics.

Methods: Thirteen patients (eight women, five men; mean age 51 ± 10 years) with severe rheumatic mitral stenosis who underwent mitral valve replacement surgery were examined on preoperative day 1, using cardiac catheterization and echocardiography. Specimens from the mitral valve and left atrium of each patient were evaluated for CD 62E and CD 62P expression using indirect immunoperoxidase and immunofluorescence techniques

Results: A great majority of patients presented E and/or P selectin expression of variable intensity on

atrial, valvular and atrial myocardial blood vessel endothelium. A more diffuse and stronger reaction for CD 62P was noted compared to that for CD 62E. The left ventricular end-diastolic diameter and left atrial diameter were positively correlated with endocardial CD 62P and CD 62E expression. Right atrial pressure was also strongly and positively correlated with endocardial expression of CD 62E ($r = 0.80$, $p = 0.03$) and CD 62P ($r = 0.8$, $p = 0.02$).

Conclusion: Marked tissue expression of CD 62E and CD 62P was identified on atrial, valvular and atrial myocardial blood vessel endothelium. Moreover, the degree of expression of adhesion molecules was significantly correlated with the left atrial and left ventricular chamber diameters, as well as right atrial pressure.

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Adhesion molecules including E-selectin and P-selectin are involved in the first step of leukocyte adhesion at sites of inflammation or injury, characterized by rolling and tethering of leukocytes to the endothelial surface, platelets, or other leukocytes (1). Adhesion molecules promote the participation of endothelial cells in inflammatory responses through the active recruitment of immune effector cells, and play a crucial role in the pathogenesis of vascular diseases (2-4). Endothelial-selectin is expressed only on activated endothelial cells, while soluble E-selectin is generated only by enzymatic cleavage from cell-surface E-selectin (5). Soluble P-selectin mediates rolling of activated platelets on activated endothelial cells and interactions of activated platelets with neutrophils and monocytes (6).

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Soluble forms of adhesion molecules have been detected in human plasma, and levels have been found to be elevated in various clinical presentations of coronary atherosclerosis (2-4). Recently, Yetkin et al. (7,8) showed that plasma levels of E-selectin were significantly higher in patients with mitral stenosis compared to controls, but were significantly decreased after percutaneous mitral valvuloplasty. Müller et al. (9) studied the tissue expression of adhesion molecules and found marked expression of intercellular adhesion molecule-1 (ICAM-1) and E-selectin on acute endocarditis and on larger portions of degenerative heart valves.

To the present authors' knowledge, there have been no investigations examining cell adhesion molecules on endothelial cells of the mitral valve in patients with rheumatic mitral stenosis. The aim of the present study was to examine tissue expression of the adhesion molecules E-selectin and P-selectin on atrial, valvular and atrial myocardial blood vessel endothelium of patients with rheumatic mitral stenosis, before they underwent mitral valve replacement surgery.

Clinical material and methods

Study population

Between 1999 and 2002, 13 patients (eight women, five men; mean age 51 ± 10 years) with severe rheumatic mitral stenosis who underwent mitral valve replacement surgery were included in the study. The demographic data of patients are listed in Table I. The history of all patients was recorded, and all underwent clinical and echocardiographic examinations. None of the patients had suffered and acute rheumatic fever attack for at least 10 years. The other exclusion criteria included immunological disease, sepsis, active infection, infective endocarditis, a history of recent infection (<3 months), history of acute rheumatic fever (<6 months), and clinical, electrocardiographic or angiographic evidence of coronary artery disease.

The investigation conformed to the Declaration of Helsinki, and was approved by regional ethics committee (10). All patients provided their written informed consent to participate in the study.

Echocardiography

All echocardiographic examinations were undertaken using a System Five cardiac ultrasound scanner (GE Vingmed Ultrasound, Horten, Norway) fitted with 2.5-3.5 MHz transducers. All patients were examined on preoperative day 1 while in the left lateral decubitus position, using precordial M-mode and two-dimensional echocardiography. The left atrial diameter, left ventricular internal cavity dimensions and left ventricular percentage fractional shortening were measured from the parasternal long-axis view. The mitral valve area was calculated using the pressure half-time method.

Cardiac catheterization

Cardiac catheterization and coronary angiography were performed using Judkin's technique via the right femoral artery.

Immunohistological evaluation

Mitral valves and the left atrial wall neighboring the mitral valve were obtained during valve replacement surgery. Care was taken to obtain valve specimens from areas without macroscopic calcification in order to ensure an intact endothelial layer. Selectin expression was assessed on the mitral valve endocardium, the atrial endocardium, and the endothelium of the atrial myocardial blood vessels. Endocardial endothelial selectin expression was scored as one after examining both mitral valve and atrium. All specimens were immediately frozen in liquid nitrogen and stored at -80°C . Cryostat sections (6-8 μm -thick) were obtained on gelatin-coated slides and held in humidity-free con-

Table I: Characteristics of patients with mitral stenosis (n = 13).

Parameter	No. of patients/ Value
Age (years)*	51 ± 10
Gender ratio (M:F)	5:8
Hypertension (n)	1
Diabetes (n)	0
Current smoking (n)	2
Atrial fibrillation (n)	12
PCWP (mmHg)*	28 ± 5
Right ventricular pressure (mmHg)*	51 ± 10
Right atrial pressure (mmHg)*	6 ± 2
Left atrial size (cm)*	5.6 ± 0.9
Mitral valve area (cm ²)*	1.3 ± 0.2
Mean mitral gradient (mm Hg)*	7 ± 3
LV end-diastolic diameter (cm)*	5.3 ± 0.6
LV end-systolic diameter (cm)*	3.4 ± 0.4
Fractional shortening (%)*	36 ± 6
Pulmonary artery pressure (mmHg)*	45 ± 9

*Values are mean \pm SD. LV: Left ventricular; PCWP: Pulmonary capillary wedge pressure.

tainers at room temperature until evaluation. Conventional histological examination was performed on representative tissue sections stained with methylene blue to evaluate structural integrity and cellular infiltration prior to immune labeling. The indirect immunoperoxidase and indirect immunofluorescence (fluorescein isothiocyanate; FITC) procedures used have been described in detail previously (11). In brief, sections were fixed in cold acetone for 10 min and air-dried for at least 30 min. They were then incubated with monoclonal IgG antibodies against mouse E-selectin (CD 62E) (1:50) (#M7105; Dako, USA) and P-selectin (CD 62P) (1:50) (#M 7199; Dako) for 60 min at room temperature in humidity chambers. After washing with 0.01 M phosphate-buffered saline (pH 7.4), a group of sections were covered with FITC-conjugated rabbit anti-mouse IgG (1:20) (#F0261; Dako) containing 0.2% bovine serum albumin and 1% normal human serum. Other group of slides were covered with 3,3'-diaminobenzidine-tetrahydrochloride (DAB) conjugated anti-mouse envision kit (#K4006; Dako) for peroxidase activity, following the manufacturer's instructions. All antibodies were diluted in a background reducing buffer solution in 0.05 M Tris-HCl containing 0.1% Tween (#S3022; Dako). Counterstaining with propidium iodide (Anchor, USA) was performed for FITC-labeled sections, and with Mayer's hematoxylin for DAB-labeled sections. Control stainings were performed by omitting the initial primary antibody staining step and using a control

mouse IgG. Stained sections were examined in random manner by two investigators who were blinded to the protocol. For each observer all sections were evaluated at one sitting using the same microscope and the same magnification. The reported histological score was the average of these observations. Each section was graded for immune reaction on endocardial and vascular endothelium on a scale of 0 to +++ with both DAB and FITC. 0 was assigned to no immune reactivity; + to weak but continuous reactivity of endocardial (atrial and valvular) endothelium and/or endothelial lining of less than one-quarter of blood vessels in the atrial myocardium; ++ to moderate but continuous reactivity of endocardial (atrial and valvular) endothelium and/or endothelial lining of one-quarter to one-half of the blood vessels in the atrial myocardium; and +++ to intense but continuous immunostaining of endocardial (atrial and valvular) endothelium and/or endothelial lining of more than half the myocardial blood vessels. Data were documented with a Leica DMR microscope (Germany); images were captured via Leica DC500 digital camera (Germany).

Statistical analysis

All numerical variables were expressed as mean \pm SD. Spearman's test was used for assessment of correlations between continuous variables. A p-value <0.05 was considered to be statistically significant.

Results

Clinical, echocardiographic evaluation and cardiac catheterization

The clinical, echocardiographic and cardiac catheter-

ization variables are listed in Table I. Coronary angiography was normal in all patients. Three patients were in sinus rhythm, and 12 had chronic atrial fibrillation. Four patients had grade II mitral regurgitation, and four grade III.

Immunohistological examination

On morphological evaluation the mitral valves were fibrotic, with collagen-rich stroma and calcified areas. Minimal mononuclear cellular infiltration consisting of macrophages and/or lymphocytes was noted in some specimens. The great majority of patients with rheumatic mitral stenosis presented E and/or P selectin expression of variable intensity on vascular, atrial and mitral valvular endothelial lining (Fig. 1A-H). A more diffuse and stronger immune reaction for P selectin was noted at all endothelia compared to that for E selectin. Data relating to the immunostaining pattern and intensity of both E and P selectin are listed in Table II.

According to the present data, an especially strong CD 62E expression on both vascular and endocardial endothelium was in concordance with an activated endothelial cell state.

Correlation of hemodynamic and immunohistological data

The present data demonstrated a significant correlation between left ventricular end-diastolic diameter and CD 62E on vascular endothelium ($r = 0.84$, $p = 0.001$), CD 62E on endocardial endothelium ($r = 0.75$, $p = 0.03$), and CD 62P on endocardial endothelium ($r = 0.85$, $p = 0.001$) (Fig. 2). The left ventricular end-systolic diameter correlated positively with CD 62E ($r = 0.72$, $p = 0.005$) and CD 62P ($r = 0.6$, $p = 0.04$) on vascular

Table II: Selectin expression pattern and intensity of patients.*

Patient no.	CD 62E ve	CD 62E ee	CD 62P ve	CD 62P ee
1	++	+++	+++	+++
2	++	++	+++	++
3	++	++	+++	++
4	+++	+++	+++	+++
5	+	+	++	++
6	++	++	++	++
7	++	++	+++	++
8	+	+	+++	++
9	++	+	+++	++
10	+++	++	+++	+++
11	+++	+++	+++	+++
12	++	+	+++	++
13	+++	+	+++	+++
Mean \pm SD	2.15 \pm 0.69	2.00 \pm 0.82	2.85 \pm 0.38	2.38 \pm 0.51

*CD 62E: E-selectin; CD 62P: P-selectin; ee: Endocardial endothelium; ve: Vascular endothelium. Endocardial endothelial selectin expression was scored as one after examining both mitral valve and atrium.

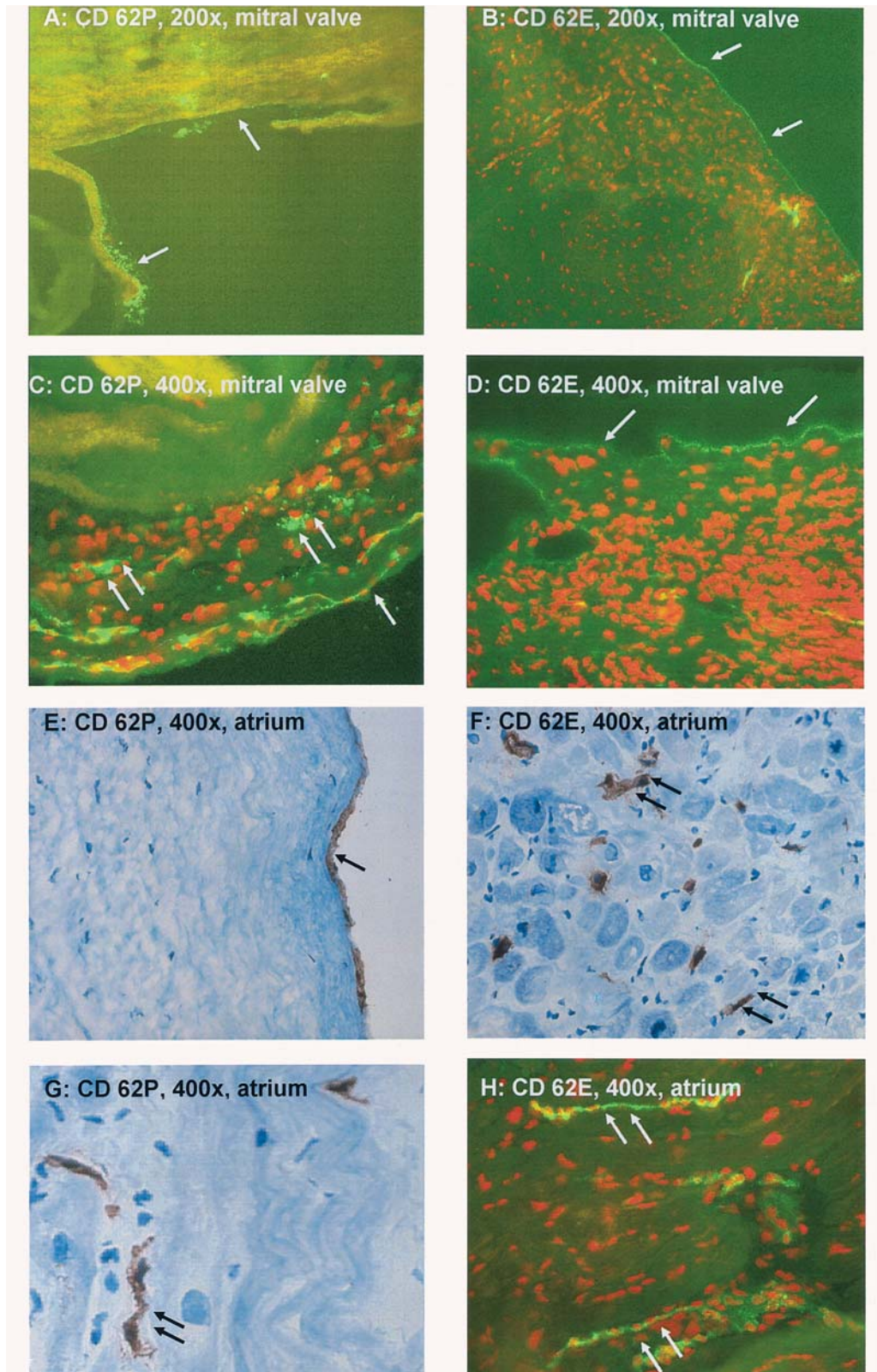


Figure 1: A, B) Low- magnification micrographs showing the CD 62P and CD 62E expression pattern on thickened fibrotic mitral valve endocardium. F-H) Small blood vessel endothelial lining exhibiting P- or E-selectin immunoreactivity observed in the atrial myocardium. Single arrows show endocardial endothelial selectin immunoreactivity; double arrows show vascular endothelial selectin immunoreactivity. Micrographs A-D and H are FITC-labeled (nuclei stained red with propidium iodide); micrographs E-G are immunoperoxidase-labeled, hematoxylin counterstained.

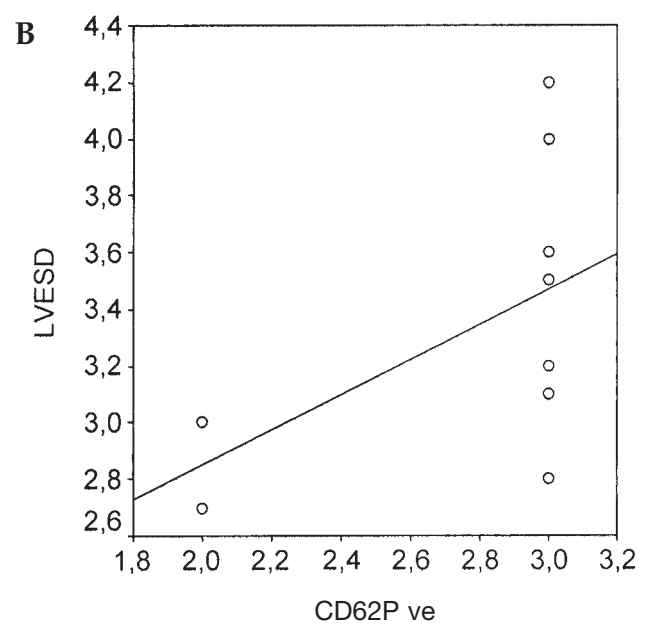
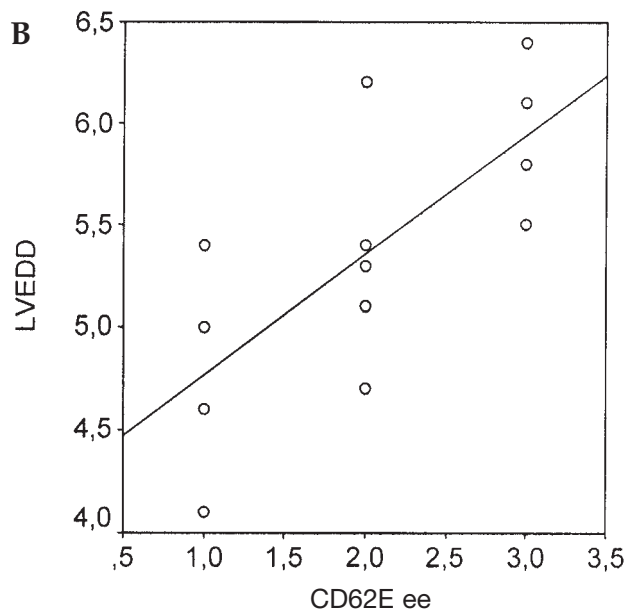
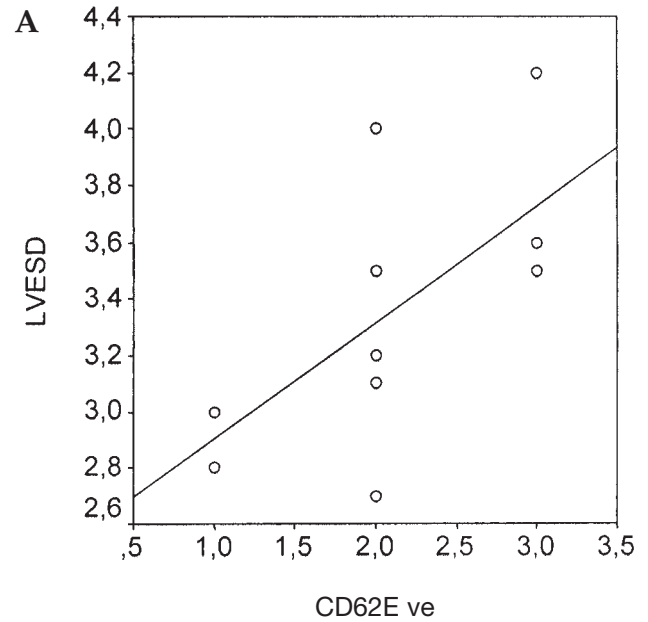
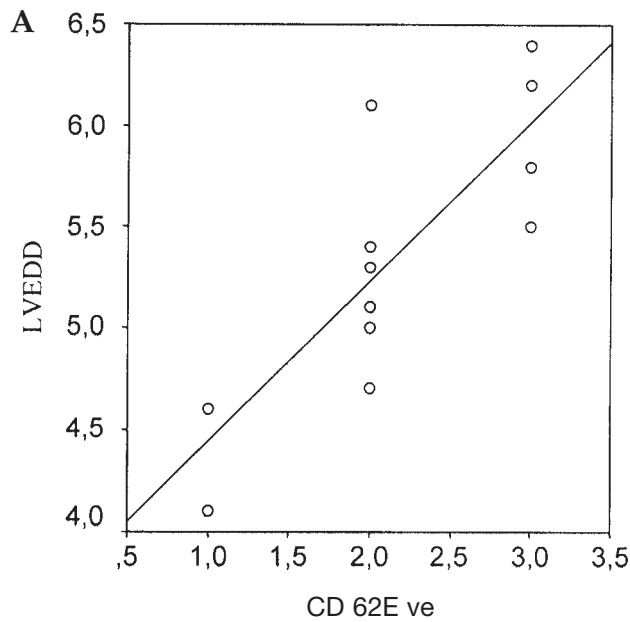


Figure 2: a) Correlation between left ventricular end-diastolic diameter (LVEDD) and CD 62E on vascular endothelium (CD 62E ve) ($r = 0.84$, $p = 0.001$). b) Correlation between LVEDD and CD 62E on endocardial endothelium (CD 62E ee) ($r = 0.75$, $p = 0.03$)

Figure 3: a) Correlation between left ventricular end-systolic diameter (LVESD) and CD 62E on vascular endothelium (CD 62E ve) ($r = 0.72$, $p = 0.005$). b) Correlation between LVESD and CD 62P on vascular endothelium ($r = 0.6$, $p = 0.04$).

endothelium (Fig. 3). The left atrial diameter correlated positively with CD 62E on vascular endothelium ($r = 0.6$, $p = 0.02$), CD 62E on endocardial endothelium ($r = 0.73$, $p = 0.01$) (Fig. 4), and CD 62P on endocardial endothelium ($r = 0.74$, $p = 0.01$). Right atrial pressure presented a strong positive correlation with both CD 62E ($r = 0.80$, $p = 0.03$) and CD 62P ($r = 0.8$, $p = 0.02$)

expression on endocardial endothelium. The right atrial pressure was not significantly correlated with atrial vascular endothelial selectin expression. Neither was any significant correlation identified between adhesion molecule expression and the severity of mitral regurgitation.

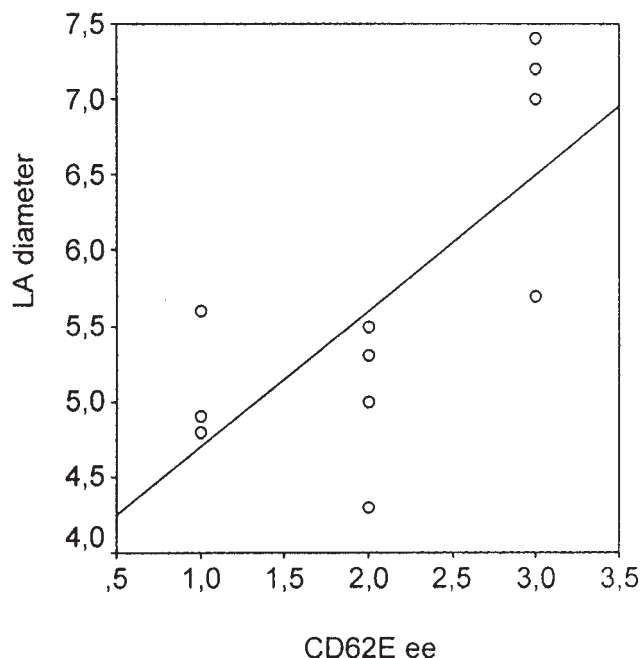


Figure 4: Correlation between left atrial (LA) diameter and CD 62E on endocardial endothelium ($r = 0.73$, $p = 0.01$).

Discussion

To the best of the present authors' knowledge, this study is the first to demonstrate marked tissue expression of the adhesion molecules E-selectin and P-selectin on atrial, valvular, and atrial myocardial blood vessel endothelium of patients with mitral stenosis before they underwent mitral valve replacement. The expression of these adhesion molecules was significantly correlated with both left atrial and left ventricular diameters.

Adhesion molecules promote the participation of endothelial cells in inflammatory responses through the active recruitment of immune effector cells. In the pathogenesis of inflammation, as soon as specific adhesion molecules are expressed on endothelial cells, granulocytes and monocytes adhere to the endothelial lining followed by transmigration across the endothelium (12). It is known that adhesion molecules differ in their antigen expression depending on their location and their state of activation (13-15).

Adhesion molecule expression can be up-regulated in response to cytokine or hemodynamic forces. The cytokines tumor necrosis factor alpha and interleukin-1 have been shown to induce production of intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1) and E-selectin (16). An in-vitro model of human umbilical vein endothelial cells exposed to steady unidirectional laminar shear stress showed a time-dependent increase in ICAM-1 expres-

sion, while VCAM-1 and E-selectin remain unchanged. In contrast, humoral (cytokine and bacterial endotoxin) activation resulted in induction of all three adhesion molecules in the same model (17,18). Ando and Kamiya (17), showed that changes in shear stress cause changes in adhesion molecule expression of vascular endothelial cells, while Gonzales and Wick (19) demonstrated that shear stress induces endothelial VCAM-1 expression and increases monocytic cell adherence. In the present study, histological examination of the tissues revealed minimal mononuclear cellular infiltration consisting of macrophages and/or lymphocytes in some specimens. Thus, the expression of selectins in the present study population was most likely due to ongoing high shear stress in the context of severe mitral stenosis, while the demonstration of adhesion molecules on atrial myocardial blood vessel endothelium was probably an indication of ongoing low-grade inflammation.

In the sera of healthy persons, soluble adhesion molecules are detectable at low levels, but these are increased in patients with various disorders, particularly those of inflammatory or vascular etiology. The levels of soluble adhesion molecules have been found to be proportional to their cell surface expression in supernatants of cytokine-activated cultured human umbilical vein endothelial cells (9). Yetkin et al. (7) found that serum levels of the soluble adhesion molecules E-selectin, ICAM-1 and VCAM-1 were increased in patients with rheumatic mitral stenosis compared to healthy controls. They also excluded patients with a history of acute rheumatic fever of less than six months' duration. Thus, the results of the present study confirm those of Yetkin et al. (7), with a demonstration of marked tissue expression of E-selectin and P-selectin on atrial, valvular and atrial myocardial blood vessel endothelium of patients with rheumatic mitral stenosis.

The rheumatic involvement of the mitral valve, together with atrial inflammation, leads to left atrial dilatation, fibrosis of the atrial wall, and disorganization of the atrial muscle bundles (20). The results of the present study showed a significant correlation between the expression of adhesion molecules and left atrial diameter, which reflects the role of inflammation in left atrial dilatation in rheumatic heart disease. However, the lack of marked inflammatory infiltrate in the tissue specimens examined raises two possibilities. First, the expression of adhesion molecules on atrial myocardial blood vessel endothelium may reflect low-grade continuing inflammation which would further dilate the left atrium. Second, expression of these molecules is stimulated by shear stress in the context of severe mitral stenosis, thus serving as markers of severity of the mitral disease. The severity of mitral

stenosis would affect the left atrial diameter, and severe cases would induce higher shear stress which could further induce adhesion molecules. Lee and Lee (21) showed ultrastructural pathological changes in the left ventricular cardiomyocytes in patients with isolated rheumatic mitral stenosis, regardless of the level of left ventricular function. Although the present patients did not show reduced left ventricular systolic function, the significant correlation between adhesion molecules and left ventricular diameter and right atrial pressure might raise the possibility of these molecules serving as markers of disease severity.

It appears that only one other study relating to the tissue expression of adhesion molecules has been reported (9), and these authors found marked expression of E-selectin and VCAM-1 on both acute endocarditis and on larger portions of the degenerative valves with calcification, but no morphological or microscopic evidence of inflammation. The study did not include patients with rheumatic heart disease, however. Shahi et al. (22) reported serum levels of ICAM-1, VCAM-1 and E-selectin to be elevated in patients with non-rheumatic aortic stenosis.

Study limitations

A major limitation of the present study, namely the lack of a control group, might have been overcome by obtaining normal tissue from autopsy cases. However, the time interval between death and obtaining tissues at autopsy would clearly alter the expression of the evaluated selectins. Muller et al. (9) used post-mortem valves (removed not more than 6 h after death) as a control group. The number of patients in the present study was also low, and this might have resulted in either non-significant (pulmonary artery pressure, pulmonary capillary wedge pressure, etc.) or less significant correlation coefficients. Thus, further studies with a control group are needed to elucidate the exact mechanism of the relationship between the expression of these molecules with hemodynamic data years after the subsidence of rheumatic valvular inflammation.

In conclusion, the results of the present study showed marked tissue expression of the adhesion molecules E-selectin and P-selectin on atrial, valvular and atrial myocardial blood vessel endothelium of patients with rheumatic mitral stenosis. The degree of expression of adhesion molecules correlated significantly with the left atrial and left ventricular chamber diameters, as well as with right atrial pressure. This study was the first in humans to demonstrate a correlation of hemodynamics with the expression of adhesion molecules.

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