SIALIC ACID, INTERCELLULAR ADHESION MOLECULE-1 AND RHEUMATOID ARTHRITIS: A STUDY ON THE ERYTHROCYTE MEMBRANE

SİALİK ASİT, İNTERSELLÜLER ADEZYON MOLEKÜLÜ-1 ve ROMATOİD ARTRİT: BİR ERİTROSİT MEMBRAN ÇALIŞMASI

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SUMMARY

We aimed to measure serum levels of soluble intercellular adhesion molecule-1 (sICAM-1) and erythroc membrane sialic acid (SA) and to investigate the correlation of these parameters with each other in patie with rheumatoid arthritis (RA), and their correlation with the disease activity.

Serum sICAM-1 level was determined with sandwich enzyme-linked immunosorband assay (ELISA) and S level with the method of Shamberger in sera from 42 patients with RA and in 30 healthy controls.

Significantly lower erythrocyte membrane SA and higher serum levels of sICAM-1 were found in patie with RA than in healthy controls (p<0.001 for both). Statistically significant negative correlation betwe sICAM-1 level and erythrocyte membrane SA concentration (r=–0.49, p<0.001) and positive correlat between sICAM-1 level and Ritchie articular index (RAI) score and CRP (r=0.32, p<0.05; r=0.44, p<0.(respectively) were observed. No significant correlation was found between sICAM-1 level and ESR, and ag and disease duration. There was no correlation between values of CRP, RAI score and ESR and erythrocy membrane SA concentration.

From these data, it is concluded that decreases in erythrocyte membrane SA concentration and increases sICAM-1, ESR and CRP levels are present in RA and that the increased sICAM-1 in RA might be due to decreased erythrocyte membrane SA concentration. The increased levels of sICAM-1 and its correlatio with other parameters may be a significant and novel marker for evaulating the disease status and th activity of RA.

ÖZET

Bu çalışma romatoid artritli (RA) hastalarda serum intersellüler adezyon molekülü-1 (sICAM-1) ve eritr membran sialik asit (SA) düzeylerini araştırmak ve bu düzeylerin hastalık aktivitesi ile ilişkisini incelem amacıyla yapıldı.

Çalışmaya alınan 42 RA'li hasta ve 30 sağlıklı kişide sICAM-1 düzeyi enzyme-linked immunosorband as say (ELISA) ile, SA konsantrasyonu ise Shamberger metodu ile tayin edildi.

RA'li hastalarda eritrosit membran SA konsantrasyonu kontrol grubuna göre anlamlı olarak düşük, sICA 1 düzeyi ise anlamlı olarak yüksek bulundu (p<0.001). SA ve sICAM-1 arasında negatif bir korelasyon var

(r=–0.49, p<0.001). Ayrıca sICAM-1 ile Ritchie Artiküler İndeksi (RAI) ve CRP arasında pozitif bir korelasy bulundu (sırasıyla r=0.32, p<0.05; r=0.44, p<0.01). Ancak sICAM-1 ile ESR, yaş ve hastalık süresi arasın anlamlı bir korelasyon bulunamadı. Ayrıca eritrosit membran SA konsantrasyonu ile de CRP, RAI ve E arasında anlamlı bir ilişki bulunamadı.

RA'li hastalarda eritrosit membran SA konsantrasyonunun azaldığı, sICAM-1 düzeyinin ise CRP ve ESR birlikte yükseldiği ve sICAM-1 düzeyindeki yükselmenin eritrosit membranındaki SA oranının azalması bağlı olabileceği sonucuna varıldı. Ayrıca hastalık aktivasyonu takibinde sICAM-1'in faydalı bir paramet olabileceği kanısına varıldı.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, multi-system autoimmune disease characterised by persistent synovitis. The chronic inflammation leads to development of a pannus-an agresssive inflammatory tissue where activated T lymphocytes, macrophages, B cells and the cytokines they produce, as well as active angiogenesis play a major part in the progressive destruction of the joints (1). Inflammation is characterized by the accumulation of leukocytes and other mesenchymal cells at sites of injury or infection. Many of the adhesive molecules mediate the interaction between endothelium and leukocyte. There are three general classes of adhesive molecules present on leukocytes and endothelium: integrins, selectins, and members of the immunoglobulin superfamily of cell surface proteins. Integrins and selectins on leukocytes mediate the adhesion of circulating cells to endothelium, whereas selectins and members of the immunoglobulin superfamily on the endothelium mediate their stickness for leukocytes (2,3). Immunoglobulin superfamily adhesive molecules involved in the cell-cell adhesion required for inflammation is the immunoglobulin superfamily. The members of this superfamily are characterized by the presence of one or more immunoglobulin homology regions, each consisting of a disulfide-bridged loop with a number of antiparallel β-pleated strands arranged in two sheets. Members of the immunoglobulin family of proteins that are involved in adhesion appear to possess predominantly H-type domains. Two members of the immunoglobulin superfamily. VICAM-1 and ICAM-1 are ligands for leukocyte integrins (4).

The soluble ICAM-1 (sICAM-1) is functionally active and has been noted in a variety of inflammatory and neoplastic conditions (5,6). sICAM-1 could compete with cell-bound ICAM-1 on endothelial cells for one or more members of the CD18 family of leukocyte adhesion molecules, thus preventing attachment or promoting deattachment and thus allowing the cells to traffic. Alternatively, sICAM-1 could act to promote transmembrane signalling to the lymphocyte by engaging leukocyte function-associated Ag (LFA-1) (7,8). Finally, sICAM-1 might simply be the consequence of inflammation, tissue damage, and nonspecific proteolysis.

Sialic acid (SA), a family of acetylated or glycosylated derivations of neuraminic acid, is widely distributed in mammals. It usually occurs as a terminal component at the non-reducing end of carbohydrate chains of glycoproteins and glycolipids. The sialic acid-rich carbohydrate chains of glycoproteins and glycolipids (2,9). The sialic acid-rich carbohydrate side chains are predominantly O-glycosidically linked oligosaccharides (10). It binds tightly to both hydroxyapatite and cells, and thus, it might serve as a cell-adhesion molecule, allowing cells to attach to the extracellular matrix (11). In the erythrocyte membrane, SA is mainly contained in the SA-rich glycophorins.

In the present study, we measured the serum levels of sICAM-1 and erythrocyte membrane SA concentration and investigated their relations in patients with RA and whether these levels were correlated with the clinical features of this disease.

MATERIAL AND METHODS

We included 42 patients with RA (8 men, 34 women, mean age: 45.78±9.21 years, range: 28-65 years), and 30 healthy subjects (20 men, 10 women, mean age: 42.73±8.44 years,

range: 25-65 years) in the study. The mean disease duration was 59.80±44.83 month (range: 5-240 month). Patients with RA were examined and the diagnosis confirmed by at least two experienced rheumatologists, according to the 1987 revised criteria of the American College of Rheumatology (12). None of the patients had received glucocorticoids within the previous 3 months. Fifteen patients were taking a combination of methotrexate and hydroxychloroquine, and 32 patients were receiving nonsteroidal antiinflammatory drugs at the time of sampling. Ten patients were not receiving any antirheumatic therapy at the beginning of this study. None of the subjects in this study was taking alcohol and had no absorption defect. The control subjects were healthy hospital personnel.

Venous blood was collected in vacutainers without additive, allowed to clot for 30 min at room temparature and centrifuged at 2000 g for 5 min. Serum aliquots were stored at –80∞C. ESR and CRP, both are the indexes of RA disease, were determined in whole blood and serum aliquots, respectively. For SA concentration, 10 ml of blood was drawn into heparinized glass tubes. SA concentration was determined in the erythrocyte membrane. Serum sICAM-1 was determined by an enzyme-linked immunosorband assay kit (Roche diagnostic, cat no: 1573659). ESR was determined according to the Westergren method and CRP by nephelometric method (Beckman Array Protein System).

Disease activity assesment

All patients had active disease, defined as the presence of at least 3 of the following features: >6 swollen joints, ESR>9.6 mg/l, morning stiffness>45 minutes duration and Ritchie Articular Index (RAI) score>10.

Membrane preparation

Erythrocyte membrane was prepared as previously described (13). The erythrocyte membranes were prepared according to Wood and Beutler (14). Red cells were separated from plasma by centrifugation at 2000xg for 10 minutes. They were washed twice with 0.9 NaCl, and 1.5 ml of packed red cells was added to a 50 ml polyethylene centrifuge tube. Fifty ml of hemolysing solution ($1x10^{-4}$ Na_2EDTA buffer (pH 7.4), and stored at –70°C until analysis. Protein concentration was determined by the Lowry method (15), with albumin as a standart (133-157 µg/100ml).

Sialic acid assay

SA concentration was determined according to the method of Shamberger (16). Briefly, 500 µl of membranes was hydrolysed with of 100 µl of H_2SO_4 (0.05 mol/l) for 1 hour, at 80°C to release SA and diluted with 400 µl of water. Then, 0.2 ml of Ehrlich's solution (0.7 g of p-dimethylaminobenzenaldehyde + 150 ml of concentrated HCL + 100 ml of distilled water) was added and vortexed. The tube was incubated in a 56°C water bath for eight hours. The tubes were vortexed gently for five hours, and gradually appeared as blue in ten-hour period. Then, 3 ml of 9 g/l NaCl solution was added to each tube and it was centrifuged at 3000 g for 15 min. The supernatant was placed in a cu-veet which was read in the spectrophotometer at 525 nm. SA concentration in supernatant was determined by a standart curve, with SA as a standard (0.5-2mmol/l).

Statistical Analysis

Statistical analyses were carried out using Student's-t test. Values are given as mean±SD. Correlations between variables were determined by Sperman's rank correlation coefficent ($p<0.05$ was regarded as significant).

RESULTS

The values of sICAM-1, CRP, ESR and erythrocyte membrane SA levels obtained from the patients with RA and healthy control subjects have been shown in Table 1. When compared with the levels in the control subjects, the values of sICAM-1, CRP and ESR in the patients with RA were significantly elevated, but erythrocyte membrane SA activity decreased. Significant negative correlations between sICAM-1 and SA were observed ($r=-0.49$, $p<0.001$; Figure 1).

Table 1. The mean values of clinical features and biochemical parameters in controls and patients

	Control subjects	Patient subjects
n	30	42
Age (years)	42.73±8.44	45.78±9.21
Duration of disease (month)	-	59.80±44.83
RAI (score)	-	38.23±11.79
ESR (mm/h)	16.23±8.88	56.90±17.56*
CRP (mg/l)	1.44±0.58	26.35±6.55*
sICAM-1 (ng/ml)	231.88±24.78	496.88±64.47**
SA (mmol/l)	1.346±0.31	0.942±0.27**

Data are mean ± SD; Comparisons are made between control and patient subjects; RAI=Ritche articular index; ESR= Erythrocyte sedimentation rate; CRP= C-reactive protein; sICAM-1= Soluble intercellular adhesion molecule-1; SA= Eryhrocyte membrane sialic acid; NS= not significant .

* p<0.0001, ** p<0.001

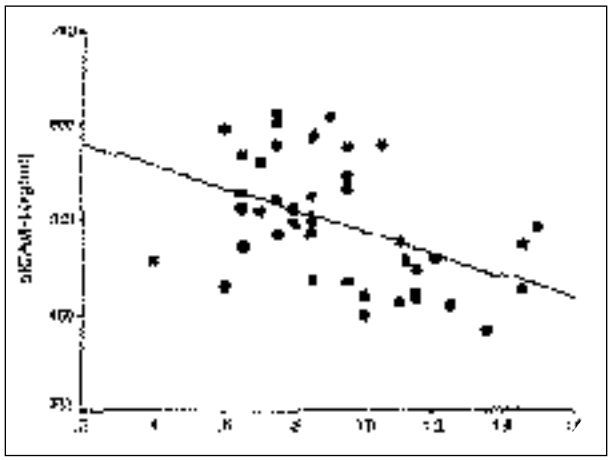

Figure 1. The relationship between eryhrocyte membrane sialic acid concentration and serum sICAM-1 levels in rheumatoid arthritis. Statistical analysis was performed using Sperman's rank correlation coefficent (r = –0.49, p<0.001).

In the RA patients chosen for this study, serum sICAM-1 levels were compared with a variety of clinical variables and measures of disease activity, including RAI score, ESR and CRP. There was a weak, positive correlation between sICAM-1 levels and RAI scores (r=0.32, p<0.05) and CRP levels (r=0.44, p<0.01). However, the presence of increased sICAM-1 levels was not correlated with ESR, age and disease duration. There was no correlation between CRP, ESR, age, disease duration and RAI score with erythrocyte membrane SA activity.

DISCUSSION

RA is a chronic sistemic disease altough its major clinical consequence is inflammation of the joints and contigous structures. A diverse system of adhesion molecules and adhesion receptors participates in orchestrating vital biologic phenomena, such as embriogenesis, cell growth and differentation, and wound repair. It appears that the main sources of sICAM-1 in vivo are mononuclear cells and endothelial cells because it was demonsrated that sICAM-1 released by activated mononuclear cells and by activated endothelial cells in vitro (17,18,19).

To date, the release of sICAM-1, by secretion or shedding, has been well documanted with many reports of elevated levels in disease (20). The current studies were prompted by recent observations that sICAM-1 could be detected at increased concentrations in patients with inflammatory disease, including sjogren's syndrome (21), familial mediterranean fever (22), connective tissue disease (23) and coronary artery disease (24). In the case of RA and other inflammatory joint diseases, studies have assessed one or several sICAMs levels relative to healthy controls. We demonsrated that serum levels of sICAM-1 were significantly higher in patients with RA than in healthy control subjects. Morever, serum sICAM-1 was significantly correlated with the presence and the severity of RA. It could be postulated that elevated serum levels of sICAM-1 in patients with RA may reflect the elevated release of this molecule from a feature of the patients. In our opinion, this increase might be due to either increased sICAM-1 synthesis or induced by inflammatory cytokines such as IL-1, TNF-α and IFN-γ on the release of this molecule. Several studies have shown that the shedding of sICAM-1 is induced by activation signals and cytokines such as IL-1, TNF-α and IFN-γ (17,25). sICAM-1 may have a pivotal role in inflammation. Previously, no correlation was found between plasma levels of sICAM-1 with disease markers ESR and

CRP (26). Elsewhere, serum levels of sICAM-1 have shown a weak correlation with ESR in patients treated with non-steroidal anti-inflammatory drugs (27). In our study, we found that sICAM-1 had a significant association with CRP and RAI, but not with ESR. It could be postulated that the serum sICAM-1 level may reflect the level of inflammation in patients with RA.

In the present study, decreased erythrocyte membrane SA concentration was found in RA patients compared with the control group, and a significant negative correlation was found between erythrocyte membrane SA concentration and sICAM-1 level. Terminal SA residues of glycoproteins and glycosphingolipids are important in blood clot formation, interaction of hormones with their target cells, neurotransmission, cell-cell interactions, and cellular transformation (28). Cell-cell interactions may also lead to the formation or impaired breakdown of other mediators of inflammation. Serum SA concentrations were reported to be elevated in a number of malignant conditions (29,30). It is speculated that they reflect the release and accumulation of tumor products. It is also known that an increased concentration of acute phase reactans caused by an acute inflammatory disease or by an injury can cause increased serum SA concentrations (31). Therefore, sICAM-1 levels may have increased with damage of the cell membrane. Our study demonsrated that SA concentration of erythrocyte membrane was in low in patients with RA. This decrease might be due to either decreased SA synthesis or increased sialidase activity. Some investigators have claimed that erythrocyte membrane deformation is accompained by desialylation of membrane glycoconjugates caused by cleaving of terminal SA residues or by removal of sialoglycoconjugates (32-35). The reduction of erythrocyte membrane SA concentration may be related to qualitative alterations of the cell membrane. In our opinion, reduced SA concentration in membrane may be secondary to altered membrane functions, including adhesive properties, and altered signal transmission then serum sICAM-1 level was increased. Further studies are in progress to eludicate the mechanisms of this phenmenon. An understanding of the role of membrane SA in mediating the initial events of inflammation may lead to new modes of therapy for the rheumatic diseases.

From these data, it is concluded that decreases in erythrocyte membrane SA concentration and increases in sICAM-1, ESR and CRP levels are present in RA and that the increased sICAM-1 in RA might be due to the decreased erythrocyte membrane SA concentration. The increased levels of sICAM-1 and its correlations with other parameters may be a significant and novel marker for evaulating the disease status and the activity of RA. Further studies are needed to document the conditions of release and the potential immunoregulatory role of sICAM-1 in RA.

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