

## SOLUBLE P-SELECTIN IN PRIMARY SJÖGREN'S SYNDROME

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**Purpose:** Sjögren's syndrome (SS) is characterized by focal lymphocytic infiltration of the salivary, lacrimal, and other exocrine glands. P-Selectin is a cell adhesion molecule that plays critical roles in the homing of the inflammatory cells to the site of inflammation. A soluble form of P-selectin can also be found in the plasma as a circulating protein. The sP-Selectin is also active and could play anti-inflammatory functions by preventing leukocyte-endothelium interactions. In this study, we investigated serum levels of sP-Selectin in patients with primary SS.

**Methods:** Eight patients with primary SS (7 female, 1 male; mean age 44.88 years (35-58)) and 10 healthy subjects (6 female, 4 male; mean age 32.3 years (19 to 44)) were enrolled in this study. sP-Selectin levels were measured using the quantitative sandwich immunoassay technique.

**Results:** The mean sP-Selectin levels were 168±94.7 ng/mL and 50.7±29.0 ng/mL for patients with SS and for healthy controls, respectively. sP-Selectin levels were significantly higher in SS patients than in the healthy controls (p<0.01).

**Conclusion:** The preliminary data from this pilot study suggested that sP-Selectin might have some place in the pathobiology of SS.

**Key Words:** Sjögren's Syndrome, Soluble P-Selectin, Platelet, Endothelium.

### PRİMER SJÖGREN SENDROMUNDA ÇÖZÜNÜR P-SELEKTİN DÜZEYLERİ

**Amaç:** Sjögren sendromu (SS) tükürük, lakrimal ve diğer ekzokrin bezlerin fokal lenfositik infiltrasyonu ile karakterizedir. P-selektin inflamatuvar hücrelerin inflamasyon alanına yerleşmesinde kritik roller oynayan bir hücre adezyon molekülüdür. P-selektin kan dolaşımında çözünür bir formda da bulunabilir. Çözünür p-selektin aktif bir moleküldür ve lökosit-endothel etkileşimini önleyerek anti- inflamatuvar fonksiyonlar gösterebilir. Bu çalışmamızda primer SS hastalarda çözünür p- selektin düzeylerini inceledik.

**Metodlar:** Çalışmaya primer SS tanısı almış 8 hasta [7 kadın, 1 erkek; ortalama yaş 44.88 yıl (35-58)] ve 10 sağlıklı kontrol [6 kadın, 4 erkek; ortalama yaş 32.3 yıl (19- 44)] dahil edildi. Çözünür p-selektin düzeyleri kantitatif sandviç immünassay yöntemi ile ölçüldü.

**Sonuçlar:** Ortalama çözünür p-selektin düzeyleri SS hastaları ve sağlıklı kontroller için sırasıyla 168±94.7 ng/mL ve 50.7±29.0 ng/mL idi. Çözünür p-selektin düzeyleri SS hastalarında kontrollere göre anlamlı olarak daha yüksek idi (p<0.01).

**Sonuç:** Bu pilot çalışmada gördüğümüz ön bilgiler çözünür p-selektinin SS patobiolojisinde rol oynayabileceğini düşündürmektedir.

**Anahtar kelimeler:** Sjögren sendromu, çözünür p-selektin, platelet, endothel

Sjögren's syndrome (SS) is a systemic autoimmune disease of unknown etiology. Although the precise underlying mechanisms remain to be established, pathological specimens of patients with SS demonstrate characteristic focal lymphocytic infiltration of the salivary, lacrimal, and other exocrine glands. P-Selectin, a member of the Selectin family of cell surface receptors, is a cellular adhesion molecule that is constitutively present in the Weibel-Palade bodies of the endothelial cells and in the alpha granules of platelets (1,2). P-Selectin plays critical roles in the migration of lymphocytes into tissues (3,4). A soluble form of this p-Selectin adhesion molecule does exist in the plasma (5). Soluble p-Selectin (sP-Selectin) is increased in various connective tissue diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and systemic sclerosis (6-8). In this study we aimed to investigate serum levels of sP-Selectin in patients with primary SS in comparison to healthy controls.

### Patients and Methods

Eight patients with primary SS (7 female, 1 male, ranging in age from 35 to 58; mean 44.88 years) were enrolled in this study. All patients had keratoconjunctivitis sicca symptoms, along with at least one of the following abnormalities: 1. Positive Schirmer's test; 2. Lymphocytic infiltrate in the salivary gland obtained by lip biopsy 3. Positive serology for ANA or SsA-SsB antibodies. The mean duration of symptoms was 3.9 years (ranging from 6 months to 20 years). All of the patients were receiving hydroxychloroquine 200 mg/day at the time of blood sampling. Ten healthy subjects (6 female, 4 male, ranging in age from 19 to 44; mean 32.3 years) were also included in the study as controls. Blood sampling was performed in the morning after 15 hours of fasting. sP-Selectin levels were measured using the quantitative sandwich immunoassay technique with a commercially available assay (The Parameter Human SP-Selectin Immunoassay, R&D Systems Inc., Minneapolis, USA). Briefly, a monoclonal antibody specific for sP-Selectin was pre-coated onto a microtiter plate. Standards, samples and control were pipetted into the wells, together with a polyclonal antibody specific for sP-Selectin conjugated to horseradish peroxidase. After the removal of unbound conjugated antibody a substrate was added and color developed, which was proportional to analyte concentrations. The sP-Selectin level was determined from the standard curve constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis.

The Statistical Package for the Social Sciences (SPSS), v. 10.0 for Windows, was used to analyze the data. A non-parametric Mann-Whitney U test was performed.

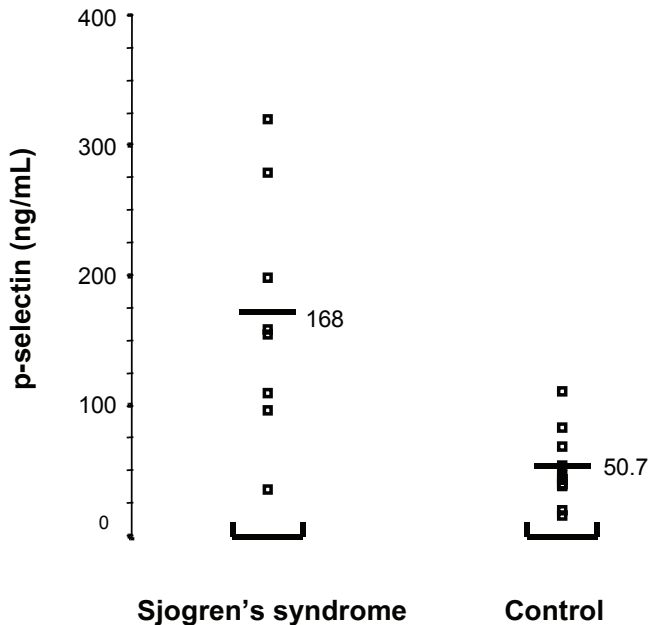
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## RESULTS

sP-Selectin levels were  $168 \pm 94.7$  ng/mL and  $50.7 \pm 29.0$  ng/mL for patients with SS and for healthy controls, respectively (Fig.1). The values are expressed as mean  $\pm$  SD. Levels of sP-Selectin were significantly elevated in SS patients ( $p < 0.01$ ).



**Figure 1:** sP-Selectin levels in patients with primary Sjögren's syndrome and healthy controls.

\*horizontal lines indicate mean values.

## DISCUSSION

Inflammatory conditions are characterized by the migration of proliferating leukocytes from the circulating blood to the tissue. Adhesion to the vascular endothelium is the first step for the homing of inflammatory cells to the site of inflammation. The adhesion process is mediated by the action of the cell adhesion molecules (9). Since lymphocytic infiltration of salivary, lacrimal and other exocrine glands is a characteristic feature of SS, special interest has been paid to the roles of adhesion molecules in the pathobiology of SS (9). However, investigations into adhesion molecule pathophysiology in primary SS have usually focused on characterizing adhesion molecule expression in salivary gland biopsy specimens (10,11).

The P-Selectin molecule is constitutively localized in the Weibel-Palade bodies of the endothelial cells and in the alpha granules of platelets (1,2). This molecule is translocated to the cell surface after activation of those cells (12-16). A soluble form of P-selectin can also be found in the plasma as a circulating protein (5). In healthy individuals, sP-Selectin is thought to originate from the alternatively spliced form found in the endothelial cells and the platelets (17). sP-Selectin can also be proteolytically shed into the circulation from the plasma membrane shortly after activation. Therefore, increased levels of sP-Selectin could reflect the activation of platelets and/or

endothelial cells (12-16). We and others have reported that sP-Selectin was elevated in a number of connective tissue disorders, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and systemic sclerosis (6-8). However, although SS could occur during the clinical course of those diseases, sP-Selectin has not been previously explored in SS. In this study, we demonstrated for the first time that serum levels of sP-Selectin were significantly higher in patients with primary SS compared to healthy controls.

An increment in sP-Selectin might reflect endothelial and/or platelet activation in SS. Expression of adhesion molecules including P-Selectin in the vascular endothelial cells in the labial salivary glands of patients with SS suggests that the source of the increased sP-Selectin concentration could be the activated endothelium (10). However, the number of P-Selectin positive vessels from the patients was not different. Moreover, sialyl Le-x, a ligand of P-Selectin, was not expressed on the infiltrating lymphocytes (10). On the other hand, there are some data regarding the contribution of platelets to the inflammatory cascade of SS. Platelets accumulate intravascularly in the inflamed salivary glandular areas in patients with primary SS (18,19). Platelet aggregation upon stimulation with epinephrine, ADP and collagen was enhanced in SS patients (19). Likewise, the platelet-specific release product beta-thromboglobulin was shown to be increased in a subgroup of SS patients and not in any of the controls (18,19). Hence, the activated platelets might be the source of elevated serum levels of sP-selectin in SS patients.

sP-Selectin is potentially active because only the lectin and epidermal growth factor domains are required to bind its receptor (20). sP-Selectin could play anti-inflammatory functions by preventing leukocyte-endothelium interactions (21,22). Moreover, interactions of neutrophils with sP-Selectin inhibited the generation of superoxide radicals (22). Therefore, apart from being a soluble marker of platelet activation, sP-Selectin might also have some regulatory roles in the pathobiological processes of SS.

The preliminary data from this pilot study suggested that sP-Selectin might have some place in the pathobiology of SS. The major limitation of our study is the limited numbers of subjects in the study group and in the control group. Moreover, a diseased-control group is also necessary. Hence, future studies with increased numbers of patients and age- and sex-matched healthy controls are needed to allow more dependable conclusions.

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