ORIGINAL ARTICLE

SERUM SOLUBLE ADHESION MOLECULES (sICAM-1, sVCAM-1, sE-SELECTIN) AND NEOPTERIN IN PATIENTS WITH SJÖGREN'S SYNDROME

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Summary: Sjögren's syndrome is a systemic autoimmune disease characterized by focal lymphocytic infiltration of the salivary and lacrimal glands. Expression and up-regulation of adhesion molecules and activation of cellular immune system is essential for the migration of inflammatory cells into tissues. Soluble forms of adhesion molecules sICAM-1, sVCAM-1, sE-selectin and neopterin were analyzed in serum of 17 patients with primary Sjögren's syndrome and 11 patients with secondary Sjögren's syndrome together with 26 age-matched healthy blood donors. There were significantly higher serum concentrations (mean ± 1SD) of sICAM-1 (362.0 ± 67.9 ng/ml, $p<0.001$), sE-selectin (78.7 ± 28.1 ng/ml, $p<0.001$) and neopterin (17.9 ± 6.4 nmol/l, $p<0.001$) in primary Sjögren's syndrome patients in comparison to control group (sICAM-1: 128.3 ± 46.9 ng/ml, sE-selectin : 46.3 ± 39.5 ng/ml, and neopterin : 7.6 ± 2.3 nmol/l). Sera from patients with secondary Sjögren's disease contained significantly higher levels of sICAM-1 (356.0 ± 62.4 ng/ml, $p<0.001$), sE-selectin (65.5 ± 27.0 ng/ml, $p<0.05$), and neopterin (18.8 ± 9.8 nmol/l, $p<0.001$) in comparison with control group. There were no significant differences between patients with primary and secondary Sjögren's syndrome in any parameters tested. No statistically significant differences in serum levels of sVCAM-1 were found either in patients with primary or secondary SS compared to control group.

Key words: *Neopterin; sE-selectin; sICAM-1; Sjögren's syndrome; sVCAM-1*

Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by a progressive lymphocytic and plasma cell infiltration of the salivary and lacrimal glands leading to the xerostomia and xerophthalmia (sicca complex) (1,19,35). SS is associated with the production of rheumatoid factors (RF) and a variety of antinuclear autoantibodies among them autoantibodies against SS-A/Ro and SS-B/La are the most important. SS displays a broad clinical spectrum extending from autoimmune exocrinopathy to the extraglandular (systemic) disease affecting the lungs, kidneys, blood vessels and muscles. SS can occur alone (primary) or in association with other autoimmune diseases (secondary) (24).

The trapping of leukocytes from the blood stream and their subsequent rolling along the activated endothelial cell lining of postcapilary venules are the earliest signs of inflammation. Rolling is an essential element of the multistep cascade leading to the leukocyte recruitment into sites of inflammation. It is mediated by the interactions between E-selectins on the surface of activated endothelial cells and their ligands, which are heavily glycosylated surface molecules of leukocytes e.g. CD15 molecule of granulocytes. Granulocytes, monocytes and a subset of memory T cells could be bound through this interaction. Next step is mediated through the interaction between adhesion molecules belonging into the families of immunoglobulins and integrins. ICAM-1 will bind cells expressing the beta2 integrins, including lymphocytes, granulocytes, and monocytes. VCAM-1 will bind only those cells expressing the beta1 integrin VLA-4; lymphocytes, eosinophils, basophils, and monocytes (11,16,21,37).

Membrane adhesion molecules are shed into the body fluids by the proteolytic cleavage or by alternative splicing on the level of mRNA (15). The elevated levels of soluble adhesion molecules are found in the serum of patients with various inflammatory diseases, and may provide some useful diagnostic or prognostic informations (14).

Neopterin is produced by macrophages after activation with interferon gamma, a cytokine produced by CD4+ helper-inducer lymphocytes. Increased neopterin concentration thus reflects the activation of specific cellular immunity. An elevation of serum or urine neopterin levels has been proven in autoimmune disorders including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, or sarcoidosis (13,39).

We found significantly higher serum levels of neopterin, sICAM-1, and sE-selectin in patients with both primary and secondary SS in comparison with healthy controls in this study.

Material and methods

Patients

This study was approved by the Ethical Committee of Charles University School of Medicine, Hradec Králové. Informed consent of all participants was obtained.

The serum samples were drawn from 17 patients (15 females, 2 males, average age 58 years, range 41-79 years) with primary SS and 11 patients with secondary SS (11 females, average age 64, range 47 - 81 years). All patients met criteria for the classification of SS according to Vitali et al. (38). The age-matched control group consists of 26 healthy female blood donors.

Blood sampling

Peripheral blood samples were collected by venipuncture into sterile tube (Sarstedt, Nuremberg, Germany). Samples were left for 1.5 hours at room temperature. Serum samples were stored frozen at -20oC and thawed only once immediately before processing.

Measurement of adhesion molecules

Serum concentrations of sICAM-1, sVCAM-1 and sE-selectin were obtained by use commercial ELISA kits Parameter Human Soluble ICAM-1, Parameter Human Soluble VCAM-1 and Parameter Human Soluble E-Selectin manufactured by RD Systems, Minneapolis, MN, USA. Serum levels of neopterin was measured by ELISA using a commercial kit (IBL, Germany). All ELISA techniques were performed according to the manufacturer's instructions. All measurement were done with the same batch, and in a duplicate.

Statistical analysis

The analysis was done using SigmaStat 2.0 statistical software Jandel Corporation, USA. The data were tested for normality. The differences between groups were calculated by t-test or Mann-Whitney Rank Sum test.

Results

sICAM-1: Statistically significant differences ($p<0.001$) in the levels of serum sICAM-1 were found in both groups of SS patients with primary SS (362.0 ± 67.9 ng/ml) and secondary SS (356.0 ± 62.4 ng/ml) in comparison with controls (128.3 ± 46.9 ng/ml)(Fig. 1.).

sE-selectin: Serum levels of sE-selectin were significantly elevated in patients with primary SS (78.8 ± 28.1 ng/ml, $p<0.001$) in comparison with controls (46.3 ± 39.5 ng/ml) as well as significant increase in concentrations of sE-selectin was observed in patients with secondary SS (65.5 ± 27.1 ng/ml, $p<0.05$) in comparison to the control group (Fig. 4.).

sVCAM-1: No statistically significant differences in serum levels of sVCAM-1 were found either in patients with primary or secondary SS compared to control group (Fig. 3.).

neopterin: Sera from patients with primary SS contained more neopterin (17.9 ± 6.4 nmol/l, $p<0.001$) than control group (7.6 ± 2.3 nmol/l). There were also significant differences in serum levels of neopterin between patients with secondary SS and control group (18.8 ± 9.8 nmol/l) (Fig. 2.).

There were no significant differences between groups of patients with primary and secondary SS in any parameter tested.

Highly significant correlations were observed in patients with SS: between serum levels of neopterin and sICAM-1 (coefficient of correlation $r=0.620$, $p<0.001$, Figure 5.), between serum levels of sICAM-1 and sVCAM-1 ($r=0.569$, $p<0.001$), and between sVCAM-1 and neopterin ($r=0,599$, $p=0.001$), but not in control group (sICAM-1 x neopterin $r=0.006$, $p>0.05$; sICAM-1 x sVCAM-1 $r=0.171$, $p>0.05$; sVCAM-1 x neopterin $r=0.035$, $p>0.05$).

Fig. 1: Comparison of serum levels of sICAM-1 in primary SS, secondary SS and control group (dash lines deliminate the interval 5% - 95% percentiles of control group).

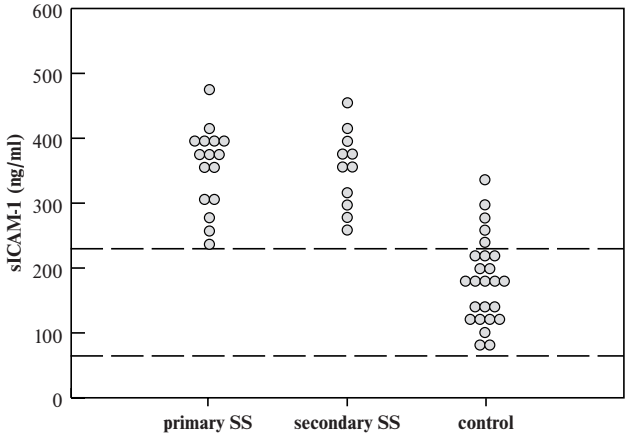


Fig. 2: Comparison of serum levels of neopterin in primary SS, secondary SS and control group (dash lines deliminate the interval 5% - 95% percentiles of control group).

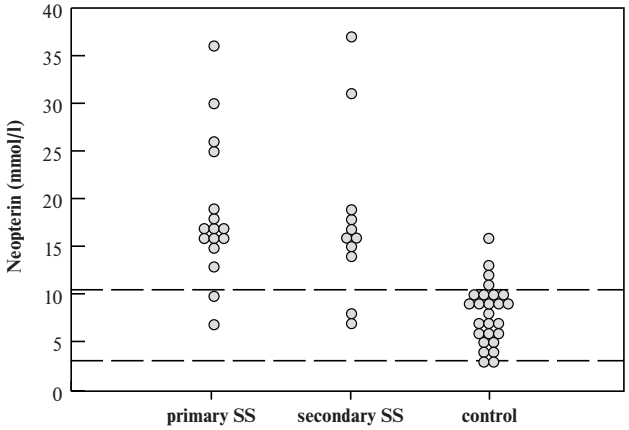


Fig. 3: Comparison of serum levels of sVCAM-1 in primary SS, secondary SS and control group (dash lines deliminate the interval 5% - 95% percentiles of control group).

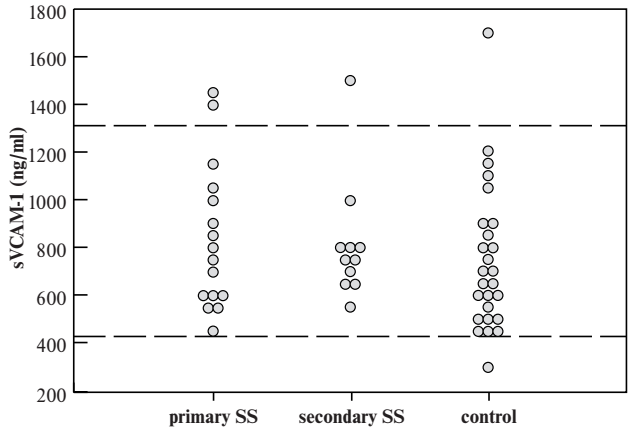


Fig. 4: Comparison of serum levels of sE-selectin in primary SS, secondary SS and control group (dash lines deliminate the interval 5% - 95% percentiles of control group).

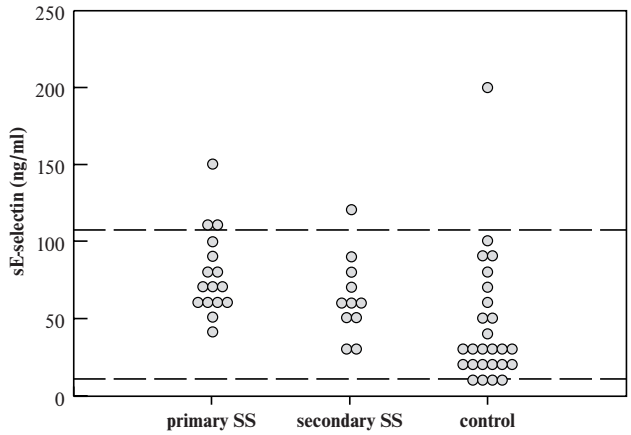
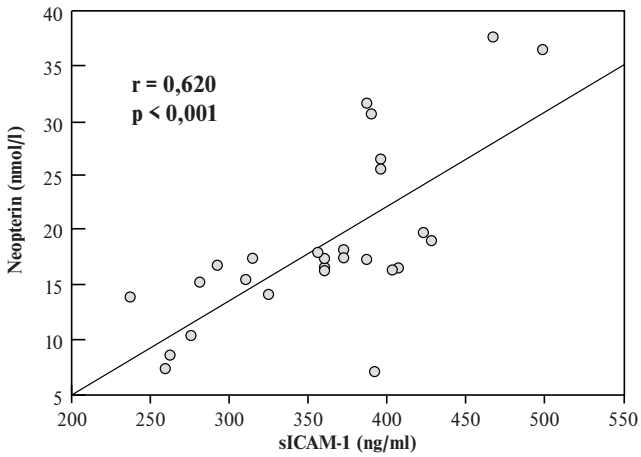


Fig. 5: Correlation between serum neopterin and sICAM-1 in group of patients with Sjögren's syndrome (r - coefficient of correlation.



Discussion

Sjögren's syndrome is a chronic inflammatory disease of unknown origin marked by inflammation and destruction of the salivary and lacrimal glands. The impairment of salivary and lacrimal gland functions is caused by the destruction of acini and ductal cells accompanied by lymphocytic infiltration, which is believed to be immunologically mediated (35). CD4+CD45RO+ memory helper-inducer T-cells are the major subsets infiltrating the exocrine glands of Sjögren's syndrome patients. B cells subsequently appear in the lesions (2).

Leukocyte adhesion is a crucial step in the development of both normal immune response and inflammation (7,16). Adhesion of leukocytes is mediated through the multiple interactions between adhesion molecules and their ligands. More recently we have learned that soluble isoforms of these adhesion structures can be found in the circulation and their levels can serve as a surrogate marker of disease activity (14,32). The information obtained from measurement of soluble adhesion molecules can be interpreted at several different levels. The expression and subsequent release of soluble cellular adhesion molecules is mediated by proinflammatory stimuli both exogenous and endogenous origins such as endotoxin, histamin, thrombin, and various cytokines. Soluble cellular adhesion molecules may be regarded simply as markers of the presence and intensity of inflammation. The clinical utility of monitoring levels of soluble adhesion molecules is not yet established, but it is supposed that the availability of commercial assay kits should allow their evaluation in many clinical settings (15).

We found significantly elevated serum levels of sICAM-1, and sE-selectin in serum of both primary and secondary SS patients in comparison with healthy control. These findings could reflect hyperactivation of the immune system of SS patients which is well documented (35). The hallmarks of SS are hypergammaglobulinemia, presence of autoantibodies as the results of polyclonal activation of B cell system which is under the control of T cell. The putative autoantigen (or autoantigens) in salivary glands of SS patients has to be presented to T cells after binding to HLA class I or II molecules. These specific interactions that are accompanied by the costimulatory and accesory interactions lead to the production of cytokines upregulating the expression of adhesion molecules in affected tissues. It has to be stressed that there are numerous sources of such cytokines in salivary glands, including macrophages, epithelial and endothelial cells, T and B cells and fibroblasts. It could not be possible to distinguish among contributions of particular cell type from the serum levels of soluble adhesion molecules. Saito et al. (30, 31) reported that the expression of ICAM-1 in salivary glands of patients affected by SS is higher than in normal salivary gland. This upregulation of ICAM-1 correlated with the intensity of T cell infiltration, which are the only source of interferon gamma enhancing the expression of ICAM-1. Johanssen et al. (18) found hig-

her levels of ICAM-1 in both serum and residual saliva of patients with primary SS in comparison with control. Recently, we have reported that serum levels of ICAM-1 is significantly higher in primary and secondary SS patients and in the case of secondary SS the level of sICAM-1 is correlated with the level of beta-2 microglobulin (20).

In comparison with ICAM-1 expression, the informations about another adhesion molecule, E-selectin, in SS are sparse. E-selectin is stored in intracellular granules of endothelial cells and it is expressed within minutes on their membranes after different stimuli including proinflammatory cytokines, products of blood coagulation, mechanical shear stress and many exogenous stimuli such as microbial products. E-selectin molecules mediate the rolling of activated granulocytes on the surface of activated endothelial cell lining through the interaction between N-terminal end of E-selectin which is lectin-like and specific sugar residues on CD15 molecule of granulocytes (40). This seems to be not so much relevant for the pathogenesis of SS, because granulocytes play only a limited role in this disease. But E-selectin could mediate the binding of particular lymphocyte populations, for example skin-homing T cells (27) and memory T cells (33). Because the lymphoid infiltration of salivary glands in SS consists of predominantly helper T cells with memory phenotype (CD4+CD45RO+) one could speculate, that E-selectin is partially responsible for this accumulation, but Aziz and coworkers (3) has not found increased expression of E-selectin in salivary gland of SS patients. Their results are in agreement with the findings of Flipo and coworkers (12). The role of E-selectin in SS warrants further investigation.

Our findings that serum levels of E-selectin are increased in both primary and secondary SS patients in comparison with control may reflect overall activation of endothelial cells in these patients. The serum or plasma levels of E-selectin are reported to be elevated in patients with other immunopathological diseases for example atopic dermatitis and psoriasis (9), vasculitis and scleroderma (8), as well as in malignancies (4,36), and in infectious complications such as septic shock (26). Spronk et al. (34) have not found elevation of serum E-selectin in patients with systemic lupus erythematosus and there was no correlation of sE-selectin with disease activity. Our results suggest that elevated serum levels of sE-selectin in primary and secondary SS are caused by the activation and probably destruction of endothelial cells (5,6). This destruction is partially mediated by the autoantibodies against endothelial cells which are seen in SS patients (22). It is interesting that there is no difference in the level of E-selectin between primary and secondary SS patients.

The serum levels of sVCAM-1 were not elevated in SS patients in comparison with control in our study. VCAM-1 is expressed on the surface of activated endothelial cells and it is responsible together with ICAM-1 for the firm adhesion of leucocytes. There is a great difference in the action of VCAM-1 and ICAM-1. In the case of ICAM-1 all leucocytes could adhere through the LFA-1 beta2 integrin which is expressed on all leucocytes. In a sharp contrast, in the case of VCAM-1 the adherence of leucocytes is much more selective because VCAM-1 is a ligand for beta1 integrin VLA-4. Enhanced expression of VCAM-1 on the endothelium may facilitate the transmigration of monocytes and CD4+ memory effector cells, since these cells express VLA-4, whereas „naive“ CD4+ T cells do not (17).

Neopterin is an oxidation product of 7,8-dihydroneopterin which is synthesized from guanosine triphosphate (GTP) by the enzyme GTP cyclohydrolase I. The production of 7,8-dihydroneopterin represents a first step in the pathway leading to the synthesis of tetrahydrobiopterin, an enzyme cofactor. For some yet unknown reason the activity of GTP cyclohydrolase I is present in a great excess compared to the enzymes active distally in the tetrahydrobiopterin pathway in human macrophages after treatment with interferon gamma leading to a remarkable rise in 7,8-dihydroneopterin. 7,8-dihydroneopterin is in equilibrium with its oxidized form, neopterin, which can be monitored in body fluids (23,29,39).

Increased concentration of neopterin in serum or in urine has been documented in many autoimmune disorders, including rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, or inflammatory bowel disease, and increased neopterin levels have been associated with disease activity (10,25,28).

Numerous factors, genetical, infectious, immunological and environmental are thought to play a role in the pathogenesis of SS. Substantial progress has been made recently in the investigation of dysregulation of the immune system in SS patients. Our results support immunopathological etiology of this enigmatic disease.

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