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The Significance of Salivary and Serum Interleukin 6 and Basic Fibroblast Growth Factor Levels in Patients with Sjögren's Syndrome

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A B S T R A C T

Role of various cytokines have been implicated in the development and perpetuation of Sjögren's syndrome (SS), but no specific cytokine could be determined as a major contributor to the SS. Salivary and serum interleukin 6 (IL-6) levels have been studied previously in patients with SS, but data upon salivary and serum basic fibroblast growth factor (bFGF) in SS are lacking. The aim of this study was to evaluate levels of salivary and serum IL-6 and bFGF in 18 patients with SS, age range 32–79, mean 54.05 years. Control group consisted of 23 healthy participants, mean age 25 years. Serum IL-6 and bFGF levels were not significantly different between patients with SS and healthy controls. Elevated levels of salivary IL-6 and bFGF in patients with SS when compared to the healthy controls were found ($p<0.001$). We might speculate that higher levels of salivary IL-6 and bFGF in patients with SS might originate from local production probably having source in the salivary glands.

***Key words**: salivary cytokines, serum cytokines, Sjögren's syndrome*

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Introduction

Sjögren's syndrome (SS) is a chronic inflammatory disorder characterized by lymphocytic infiltration of lacrimal and salivary glands, which results in dry eyes and mouth[1]. Despite the extensive worldwide literature, pathogenesis of SS remains poorly understood. It has been postulated that a combination of immunologic, genetic and environmental factors are involved in the development of SS [2]. Interesting data on cytokine production from the salivary glands of SS patients have been reported in the past few years. From reviewing the published literature it is difficult to obtain a clear picture of the cytokine profile in SS. This is largely due to the major differences in techniques and specimens used in these studies. All the available data clearly suggest an *in situ* immune response in the exocrine glands of SS patients, with the epithelial cell playing the role of antigen presenting cell. As a consequence T-cells are attracted and become activated. Produced cytokines are responsible for inflammatory perpetuation and stimulation of B-lymphocytes[3].

The proinflammatory cytokine IL-6 play an important role in the inflammatory, autoimmune and malignant processes. IL-6 is generated by lymphocytes, endothelial cells, fibroblasts and epithelial cells. It is well known that collagen diseases and other autoimmune diseases are associated with elevated levels of serum IL-6[4]. Some authors[5–7] found elevated levels of salivary IL-6 in patients with SS, but data upon serum IL-6 in SS are scarce.

The role of basic fibroblast growth factor (bFGF) has been implicated in the numerous processes of normal and neoplastic tissues of the human and animal bodies. BFGF has a pleiotrophic effects and can act as a growth or differentiation factor depending on the cell type and local environment[8]. Various tissues from ectodermal, endodermal and neural origin have been shown to produce bFGF and serum bFGF levels are elevated in patients with wide spectrum of tumors[9–11].

There are few reports in the literature which evaluate serum and salivary IL-6 levels in patients with SS, but no data upon bFGF either salivary or serum in patients with SS could be found in the worldwide literature. The aim of this study was to evaluate significance of salivary and serum IL-6 and bFGF levels in patients with SS.

Materials and Methods

Prior to investigation informed consent according to FDA[12] was obtained from each participant. The patient group consisted of 18 participants, all women, with confirmed SS according to the Vitali et al.[13], revised version of the European criteria set for classification. The patients with SS were age range 32–79 years, mean 54,05 years. Control group consisted of 23 participants, 15 women and 8 men, mean age 25 who were healthy and did not take any medications 1 month prior to investigation.

Whole unstimulated saliva specimens were collected from each participant while they were sitting, into calibrated tubes for five minutes according to Wu Wang et al.[14]. Afterwards the saliva specimens were stored at –70 °C and kept until analysis. A 5 ml of fasting blood from cubital vein was taken from each participant between 7 and 9 A.M., frozen and stored at –70 °C until analysis.

For determination of salivary and serum bFGF levels ELISA (Quantikine, HS FGF basic, R&D Systems, Minneapolis, USA) was performed. Concentrations of salivary and serum IL-6 were determined using an ELISA (Milenia Biotec, Bad Nauheim, Germany). Sensitivity for bFGF was 0.22 pg/mL and for IL-6 was 1.2 pg/mL.

Statistical analysis was performed using Mann Whitney U-test and values lower than 0.001 were considered as statistically significant ($p<0.001$). The investigated parameters that did not show any significance were then analyzed by use of Mann Whitney U-test corrected for ties, i.e. all the values that were zero were excluded from the analysis.

Results

No significant differences in serum IL-6 values between patients with SS and controls could be found.

No significant differences in serum bFGF between patients with SS and controls could be found using Mann-Whitney U-test (Table 1).

Significant difference in salivary IL-6 between patients with SS and control group was found by use of Mann-Whitney U-test ($p<0.001$) (Table 2).

Significant difference in salivary bFGF values between patients with SS and control group was found when Mann-Whitney U-test corrected for ties was performed ($p<0.001$) (Table 3).

Discussion

Fox et al.[15] evaluated IL-6 in labial salivary glands in patients with SS with

TABLE 1
DIFFERENCE OF SERUM BASIC FIBROBLAST GROWTH FACTOR (bFGF) BETWEEN PATIENTS WITH SJÖGREN'S SYNDROME (SS) AND CONTROL GROUP

	DG	N	Mean rank	Sum of ranks	Mann-Whitney U-test	p
bFGF (serum)	SS	18	17.78	320.00		
	Controls	17	18.24	310.00	149.000	0.895
	Total	35				

TABLE 2
DIFFERENCE OF SALIVARY INTERLEUKIN 6 (IL-6) BETWEEN PATIENTS WITH SJÖGREN'S SYNDROME (SS) AND CONTROL GROUP

	DG	N	Mean rank	Sum of ranks	Mann-Whitney U-test	p
IL-6 (saliva)	SS	18	27.61	497.00		
	Controls	23	15.83	364.00	88.000	0.001
	Total	41				

TABLE 3
DIFFERENCE OF SALIVARY BASIC FIBROBLAST GROWTH FACTOR (bFGF) BETWEEN PATIENTS WITH SJÖGREN'S SYNDROME (SS) AND CONTROL GROUP (MANN-WHITNEY U-TEST CORRECTED FOR TIES)

	DG	N	Mean rank	Sum of ranks	Mann-Whitney U-test	p
bFGF (saliva)	SS	8	14.44	115.50	8.500	0.001
	Controls	11	6.77	74.50		
	Total	19				

oligonucleotide probes and in situ hybridization technique which demonstrated that IL-6 mRNA was found both in infiltrating lymphocytes and the epithelial cells. Furthermore, Fox et al.[5] reported that IL-1 and IL-6 mRNA were found in salivary gland epithelial cells using reverse transcriptase and polymerase chain reaction. Oxholm et al.[16] found greater staining of duct cells with IL-6, IFN-γ, and TNF-α in labial salivary glands of SS patients when compared to the normal glands. Grisisus et al.[7] found that salivary IL-6 concentration was elevated in patients with SS when compared to the healthy subjects. As seen in our study, the same authors found that higher IL-6 concentrations were associated with increased disease activity. Also, salivary levels were higher than serum levels in patients with SS, and these findings support the contention that salivary IL-6 is produced locally. Tischler et al.[6] reported that salivary IL-6 levels in the whole mixed saliva were significantly elevated in patients with primary SS when compared to the patients with dry mouth, but no significant differences in serum IL-6 were noticed between two groups. Same authors also stated that elevated salivary IL-6 levels are result of a local production. Study of Fox et al.[15] confirmed that salivary IL-6 levels were significantly elevated in the parotid saliva of patients with SS when compared to the healthy controls and patients with primary biliary cirrhosis (PBC). On the contrary, serum IL-6 were elevated in both SS and PBC patients, although those levels were less than those found in the saliva of the same patients. Sun et al.[17] stated that increased gene expression of IL-6 was found in patients with primary SS, but that the IL-6 was also found in minor salivary glands in control subjects. Authors[5–7] stated that increased salivary IL-6 levels could accurately monitor salivary gland involvement in SS patients. We have also tried to evaluate the role of either salivary and/or serum bFGF levels. The results of this study show that salivary bFGF levels in patients with SS are elevated when compared to the control group. It is interesting to note that in patients with elevated salivary bFGF a finding of more active disease together with additional systemic diseases was noticed. Five patients with more active SS and significantly elevated salivary bFGF showed clinical and/or laboratory signs of disease such as elevated erythrocyte sedimentation rate, elevated levels of C-reactive protein, decreased hemoglobin levels. Patient GZ had also recurrent herpes zoster infection together with SS. Patient SD had also brain hemangioblastoma and skin melanoma. Patient BA had elevated serum IgM together with skin tuberculosis and liver cirrhosis. Patients PM and CD had more active disease with persistent SS-A levels more than 100 RU.

Serum bFGF levels were not significantly different in patients with SS when compared to the control group. These results would implicate a local production of bFGF in patients with SS, but exact place of bFGF origin remains to be elucidated. In addition, elevated salivary bFGF might be a result of a chronic inflammation, which might play a role in the fibrosis seen in the later stages of gland involvement. Furthermore, to our knowledge no literature data upon salivary and serum bFGF exist and our results are difficult to compare. Further studies involving parotid and whole saliva in patients with SS with regard to IL-6 and bFGF levels are needed.

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ZNAČAJ SALIVARNOG I SERUMSKOG INTERLEUKINA 6 I BAZIČNOG FAKTORA RASTA FIBROBLASTA U BOLESNIKA SA SJÖGRENOVIM SINDROMOM

SAŽETAK

Pretpostavlja se da uloga citokina može biti uključena u nastanak i razvoj Sjögrenova sindroma (SS), ali do sada niti jedan specifični citokin nije naveden kao glavni faktor koji doprinosi SS. Salivarni i serumski interleukin 6 (IL-6) su već prije ispitivani u oboljelih od SS, dok podataka o salivarnom i serumskom bazičnom faktoru rasta fibroblasta (bFGF) u oboljelih od SS nema. Cilj ovog ispitivanja je bio ustanoviti vrijednosti salivarnog i serumskog IL-6 i bFGF u 18 bolesnika sa SS, starosti 32–79, prosječne starosti 54.05 godina. Kontrolna skupina se sastojala od 23 zdrava ispitanika, srednje dobi 25 godina. Serumske vrijednosti IL-6 i bFGF se nisu značajno razlikovale u oboljelih od SS u odnosu na kontrolnu skupinu. Povišene vrijednosti salivarnog IL-6 i bFGF su ustanovljene u oboljelih od SS u odnosu na kontrolnu skupinu ($p<0.001$). Možemo zaključiti da povišene vrijednosti salivarnog IL-6 i bFGF u oboljelih od SS-a mogu biti posljedica lokalne proizvodnje, vjerojatno unutar žlijezda slinovnica.