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## LETTER TO THE EDITOR

# Salivary interleukin-6 and tumor necrosis factor alpha in patients with drug-induced xerostomia

**It is well known that cytokines are involved in the homeostasis of oral cavity and that altered levels of either serum and/or salivary cytokines have been found in certain oral/systemic diseases. So far, cytokines in connection with xerostomia have been investigated in patients with Sjögren's syndrome. We wanted to find out whether drugs themselves influence salivary glands, which would result in altered cytokine level or whether xerostomia itself of different causes leads to the changes in salivary cytokine levels. Therefore, the aim of this study was to evaluate levels of salivary interleukin-6 (IL-6) and tumor necrosis factor (TNF)-α in 30 patients with drug-induced xerostomia, age range 29–84 and mean 63.9 years. Control group consisted of 30 healthy participants, age range 30–82 years and mean age 65.2 years. Enzyme-linked immunoassay was performed on commercially available kits. Statistical analysis was performed by use of Student's test. No significant differences in salivary IL-6 and TNF-α between patients with drug-induced xerostomia when compared with the healthy controls were found ($P < 0.05$). We might conclude that drugs do not induce damage to the salivary glands which could be seen in altered salivary IL-6 and TNF-α levels and that xerostomia itself, induced by drugs does not alter levels of the investigated salivary cytokines.**
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**Key words:** salivary cytokines; xerostomia

## Introduction

Cytokines are important part of saliva which regulate many healthy and diseased processes in the oral cavity. Regardless of the cause, xerostomia results in increased susceptibility to oral, dental and periodontal disease and difficulties in speech, swallowing and eating. All this is understandably quite frustrating for the patients. To our knowledge, salivary cytokines have been investigated in patients with xerostomia because of Sjögren's syndrome (SS). Grisius *et al* (1997), Fox *et al* (1998) and Tishler *et al* (1999) found increased salivary interleukin 6 (IL-6) levels in patients with SS. Additionally, we have also found increased IL-6 levels together with increased levels of basic salivary fibroblast growth factor in SS patients (Vučićević Boras *et al*, 2004). However, we aimed to find out whether, xerostomia due to other causes rather than SS induces changes in the salivary IL-6 and tumor necrosis factor α (TNF-α). Furthermore, we were interested in finding out whether, drugs themselves would result in altered salivary IL-6 and TNF-α levels. Therefore, we wanted to investigate whether, salivary cytokines, IL-6 and TNF-α are changed in patients with drug-induced xerostomia.

From the reviewing published literature upon salivary cytokines in various oral diseases, there has been a lot of suggestions that they might be useful diagnostic/prognostic indicators in certain oral diseases. Previously we have reported altered salivary cytokine levels of IL-6 and TNF-α in recurrent aphthous ulcers and leukoplakia (Brailo *et al*, 2006; Vučićević Boras *et al*, 2006). Increased levels of salivary IL-6 and TNF-α were also reported in patients with oral lichen planus (Rhodus *et al*, 2005). On the other hand, no significant difference in salivary IL-6 and TNF-α was found in patients with burning mouth syndrome when compared with the controls (Vučićević Boras *et al*, 2006).

The focus of this study was on IL-6 and TNF-α because these cytokines are extensively investigated in various oral diseases and for their potential diagnostic/prognostic use it would be useful to know whether their concentrations are influenced by salivary output or various drugs.

## Materials and methods

Prior to the investigation, written informed consent according to Helsinki II Agreement was obtained from each participant. The patient group consisted of 30 patients (16 women, 14 men; age range 29–84 years, mean 63.9 years) with drug induced xerostomia diagnosed on the basis of salivary flow measurement and 30 controls (age range 30–82 years, mean 65.2 years) who were healthy and did not take any medication 1 month prior to the study. Both groups underwent hematological blood tests such as complete and differential blood count, erythrocyte sedimentation rate and only patients with investigated parameters which were within normal range were included. In every participant periodontal status was recorded according to the World Health Organisation (1997) and patients with periodontal disease were excluded from the study. Candidal swabs were taken from each participant, placed on Sabouraud's agar and kept in the incubator for 48 h on 37°C. Diagnosis of candidiasis was made according to Budtz-Jørgensen *et al* (1975) and those patients (i.e. with more

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than 20 colonies) were excluded from the study. None of the participants were smokers. Saliva was collected by simple spitting method into calibrated containers while participants were sitting during 5 min according to Wu Wang *et al* (1995) between 9–11 AM. Salivary flow rates were determined for each participant and in the patient group, only patients with salivary flow rate below 0.2 ml $min^{-1}$ were included. The samples were then frozen and kept on −70°C until analysis. Cytokine immunoassay kits (R&D Systems, Minneapolis, MN, USA) were used to determine the concentration of IL-6 and TNF-α in the whole saliva samples (0.1 ml) both from patients and controls. The assays were conducted according to the manufacturer's directions. The concentration of total salivary protein was determined according to Bradford (1976). Briefly, 0.1 ml of the saliva sample was added to 5 ml of dye-reagent, incubated 10 min at room temperature and absorbance was measured at 595 nm. Protein concentration was read from the standard curve and expressed in mg $ml^{-1}$ saliva.

The results are expressed as mean ± s.d. Statistical analysis was performed by use of Student's test and Pearson's correlation. Values lower than 0.05 ($P < 0.05$) were considered as statistically significant.

## Results

Although there was a significant difference in the flow rates between the two investigated groups there were no differences in protein values (Table 1).

Concentrations of IL-6 and TNF-α are expressed as picogram per milligram of total protein. No significant differences in salivary IL-6 and TNF-α were found between patients with xerostomia and control group (Table 2). There was no correlation between levels of salivary IL-6 and TNF-α and patients' age (Pearson's correlation $P = -0.145$; $P = 0.32$, respectively), salivary flow rate ($P = 0.34$; $P = -0.002$, respectively) and number of medications taken ($P = -0.183$; $P = -0.1$, respectively). No differences in salivary IL-6 and TNF-α were found between men and women, neither in the patient nor in the control group (data not shown).

## Discussion

The results of this study showed no differences in salivary levels of IL-6 and TNF-α between patients with drug induced xerostomia and individuals with normal salivary output. Although one might notice very high variability of the data based on our results, the latter is not influenced by age, gender, number of medications and salivary output. Variability of the studied cytokines among individuals in both groups remains to be investigated.

Tishler *et al* (1999) reported that salivary IL-6 levels in the whole mixed saliva were significantly elevated in patients with primary SS when compared with the patients with dry mouth. To our knowledge this was the first and only study investigating salivary IL-6 and TNF-α level in patients with xerostomia due to other causes than SS. It seems that group with xerostomia due to other causes than SS had normal salivary IL-6 levels, which we also confirmed in this study. Grisius *et al* (1997), Fox *et al* (1998) and Tishler *et al* (1999) stated that increased salivary IL-6 levels could accurately monitor salivary gland involvement in SS patients. We can not agree with aforementioned authors, because it seems that salivary IL-6 is also elevated in other oral diseases such as oral lichen planus and leukoplakia, therefore it is not specific for SS (Rhodus *et al*, 2005, Brailo *et al*, 2006).

In this study drugs most frequently used were angiotensin-converting enzyme (ACE) inhibitors, anxiolytics, antihistamines, proton pump inhibitors, antipsychotics and anticholinergic drugs. As no differences between patients with drug-induced xerostomia and controls could be found we might hypothesise that drugs do not induce damage to the salivary glands which could have been seen as altered levels of investigated salivary cytokines. Additionally, it seems that xerostomia as a result of drug intake does not alter salivary IL-6 and TNF-α levels. However, because of a limited number of published studies upon salivary cytokines in patients with drug-induced xerostomia our results are difficult to compare.

At the end, our results suggest that salivary levels of IL-6 and TNF-α are not affected by individual characteristics such as gender, age, quantity of saliva and various drugs, which participants used in this study.

**Table 1** Salivary flow rates and total protein in the patient and the control group

| | *Flow rate (ml $min^{-1}$)* | *Total protein (mg $ml^{-1}$)* |
|---|---|---|
| Patients | 0.13 ± 0.063[a] | 1.31 ± 0.51[b] |
| Controls | 0.456 ± 0.069 | 1.18 ± 0.57 |

[a]Significant difference ($P < 0.05$).
[b]No significant difference ($P > 0.05$).

**Table 2** Salivary interleukin-6 (IL-6) and tumor necrosis factor (TNF)-α in the patient and the control group

| | *IL-6 (pg $mg^{-1}$)* | *TNF-α (pg $mg^{-1}$)* |
|---|---|---|
| Patients | 25.63 ± 49.84[a] | 7.80 ± 9.21[a] |
| Controls | 10.22 ± 17.96 | 16.00 ± 33.64 |

[a]No significant difference ($P > 0.05$).

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