Salivary eicosanoid concentration in patients with Sjögren's syndrome

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Abstract

Objective—To investigate eicosanoid concentrations in the saliva of patients with primary Sjögren's syndrome (SS).

Methods—Whole mixed saliva of 36 subjects was assayed for eicosanoid concentrations using a radioimmunoassay. Patients with primary SS having positive lip biopsy served as the study group; their results were compared with data from patients with dry mouth and negative lip biopsy (dry mouth group), and with a group of normal healthy controls.

Results—Concentrations of thromboxane B₂ were significantly ($p < 0.01$) increased in 18 patients with primary SS compared with 10 patients with dry mouth and eight healthy normal controls (1.95 (SD 0.51) ng/ml saliva compared with 0.52 (0.1) ng/ml and 0.3 (0.1) ng/ml, respectively). Similarly, prostaglandin E₂ concentrations were also significantly increased ($p < 0.01$) in 11 patients with primary SS compared with five patients with dry mouth and eight normal controls (3.75 (0.82) ng/ml saliva compared with 0.32 (0.1) ng/ml and 0.41 (0.1) ng/ml, respectively).

Conclusion—Salivary concentrations of eicosanoids are significantly increased in patients with primary SS, and this may prove helpful in the diagnosis of this disease.

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Sjögren's syndrome (SS) is an autoimmune disease that causes destruction of salivary and tear glands and is associated with dryness of the eyes and mouth. The aetiology of this disease is not known, but its distinguishing histopathological characteristic is progressive lymphocytic infiltration of exocrine glands and other organs.¹ Evaluation of salivary gland involvement in SS is complicated and several diagnostic tools have been proposed, including the classical labial salivary gland biopsy,² measurement of whole salivary flow,³ salivary chemistry,⁴ and various imaging techniques. Immunological analysis of the salivary glands strongly suggests that the T cells, especially those bearing the memory phenotype (CD4, Ro positive) and expressing the $\alpha\beta$ receptor, have an important role in the pathogenesis of SS.⁵ Furthermore, recent studies have shown that epithelial salivary gland cells produce large amounts of mRNA for interleukin-1 (IL-1), IL-6, and IL-10, which supports the existence of a local inflammatory process.⁶

The presence of arachidonic acid metabolites (eicosanoids) in saliva has been demonstrated.⁷ Concentrations of prostaglandin E₂ (PGE₂), PGF₂, and PGI₂ have been found to be independent of salivary flow rate and to display a circadian periodicity, peaking in the early morning hours.⁸ Metabolites of arachidonic acid have been found to be involved in the regulation of ion transport,⁹ and such disturbances have been documented in primary SS.¹⁰ We have investigated the possibility that increased concentrations of eicosanoids may be found in the saliva of patients with SS and that this may serve as a marker of a local inflammation.

Patients and methods

STUDY GROUPS

Eighteen patients with primary SS (16 women and two men) followed at the SS clinic, Department of Rheumatology, Tel Aviv Medical Centre, were randomly selected for the study. All patients were diagnosed as having SS according to the new criteria proposed by the EC study group.¹¹ All patients gave a positive lip biopsy specimen with a focus score of > 1 . None of the subjects participating in this study was taking non-steroidal anti-inflammatory drugs or corticosteroids before or during the collection of saliva or had any apparent oral, periodontal, or dental infection. Results from these patients were compared with data from gender and age matched patients with dry mouth who did not meet the EC criteria for SS and gave a negative lip biopsy specimen ('dry mouth' group), and with a group of normal healthy controls. The table presents the clinical and serological data of the three study groups.

EICOSANOID ASSAYS

Whole mixed saliva samples were collected from patients between 09:00 and 12:00 and were immediately frozen and stored at -20°C

Clinical and serological data of the study groups

	SS group (n = 18)	Dry mouth group (n = 10)	Healthy controls (n = 18)
Age (years)†	58.1 (12.3)	60.3 (16.1)	52.5 (10.1)
Sex (M/F)	2/16	1/9	1/7
ANF $> 1:80$ (%)	66	10	0
Anti-Ro antibodies (%)	55	0	0
Abnormal Schirmer test (< 5 mm) (5)	88	20	0

†Mean (SD).

ANF = Antinuclear factor; SS = Sjögren's syndrome.

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until processed. Saliva was collected by spitting without stimulation. The minimal amount of saliva necessary for analysis was 0.5 ml. The assay was carried out blindly with respect to the diagnosis and the clinical status of the patients. Concentrations of thromboxane B_2 (TxB_2) were determined in all 18 patients with SS and 10 patients with dry mouth, but PGE_2 was measured only in 11 patients with SS and five patients with dry mouth. Eicosanoids were extracted from saliva using ethanol, and concentrations of TxB_2 and PGE_2 were quantified by radioimmunoassay, as described previously.¹² The lower limit of detection for both prostanoids was 5 pg/ml.

STATISTICAL ANALYSIS

Statistical analysis was by Wilcoxon test. Significance was determined by p values of less than 0.05.

Results

Figures 1 and 2 show the results of the analyses of TxB_2 and PGE_2 in whole saliva in the three groups studied.

TxB_2 concentrations were significantly increased ($p < 0.01$) in 18 patients with primary SS compared with 10 patients with dry mouth and eight healthy normal controls (1.95 (SD 0.51) ng/ml saliva compared with 0.52 (0.1)

Figure 1 Salivary concentrations of thromboxane B_2 (TxB_2) in patients with Sjögren's syndrome (SS) and control groups. * $p < 0.01$ compared with other groups.

Figure 2 Salivary concentrations of prostaglandin E_2 (PGE_2) in patients with Sjögren's syndrome (SS) and control groups. * $p < 0.01$ compared with other groups.

ng/ml and 0.3 (0.1) ng/ml, respectively). Similarly, PGE_2 concentrations were also significantly increased ($p < 0.01$) in 11 patients with primary SS compared with five patients with dry mouth and eight normal controls (3.75 (0.82) ng/ml saliva compared with 0.32 (0.1) ng/ml and 0.41 (0.1) ng/ml, respectively). No correlation was found between eicosanoid concentrations and various clinical or serological parameters.

Discussion

The salivary glands are a major target organ of inflammation in primary SS, resulting in poor secretory function and dry mouth (xerostomia). As subjective complaints of dry mouth are non-specific and occur in about 20% of the general population, involvement of the salivary glands as part of SS requires an objective test. Several methods have been proposed for assessment of salivary involvement in SS, including measurement of salivary flow rate, imaging procedures, and sialochemistry. None of these methods was found to be sufficiently sensitive or specific to be of use in diagnosis.¹³ The presence of focal lymphocytic infiltrates composed mainly of CD4 positive T cells prompted us to seek inflammatory markers deriving from the salivary glands. These glands are suitable for the study of arachidonic acid metabolites because their product, saliva, is easily obtained for study and can be sampled by non-invasive methods.

We found a significant increase in PGE_2 and TxB_2 in patients with primary SS compared with normal controls and a dry mouth group. To the best of our knowledge, these findings are unique and have not been described before.

Increased concentrations of prostaglandins have been detected previously in patients with cystic fibrosis and in patients with a major depressive disorder.^{14 15} Both of these diseases are non-inflammatory in origin and therefore it is possible that an increased concentration of eicosanoids is no more than an epiphenomenon. One might speculate also that the increased concentrations of eicosanoids found in SS patients are a consequence of a small salivary volume; however, this is unlikely, as it has been shown that concentrations of arachidonic acid metabolites in whole mixed saliva are independent of salivary flow rate.¹⁶ The possibility that serum eicosanoids are filtered into the saliva has also been excluded.⁷

The role of eicosanoids in immune inflammation is not clear. Prostaglandins may modify immune function by increasing the concentrations of cyclic AMP in lymphocytes; opposing effects of PGE_2 on antibody production in vitro have also been described. The exact role of salivary eicosanoids is also not known, but it has been speculated that they participate in the defence mechanism of the oral cavity and in the absorption process of the gastrointestinal tract.^{9 16} It has also been shown that salivary eicosanoids may play a part in the regulation of ion transport into the saliva.⁷ A defect in such sodium ion transport has been

documented in several studies that revealed high sodium concentrations in the saliva of patients with primary SS.¹⁰ Our present results might explain these earlier reports.

In conclusion, we speculate that the increased salivary eicosanoid concentration found in patients with primary SS is a good indicator for the inflammatory process taking place in the salivary glands of these patients, and may support the diagnosis of this disease and help in the exclusion of patients who complain of dry mouth as a result of other causes.

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