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The status of lactoferrin and total iron binding capacity of human parotid saliva in Sjögren's syndrome

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ABSTRACT

Sjögren's syndrome (SS) is chronic salivary gland disorder characterized by a reduction in salivary and lacrimal secretion. Elevation in salivary lactoferrin has been reported in SS patients. Fluctuation in the iron binding capacity of lactoferrin has been associated with cellular damage.

Objective. *The purpose of this study was to compare the levels of salivary lactoferrin, total iron, and iron binding capacity in Sjögren's syndrome (SS) patients and healthy controls.*

Methods. *SDS-PAGE was used to examine the presence of lactoferrin in 102 patients and 20 healthy controls. A colorimetric assay was used to examine the level of total salivary iron and iron binding capacity in patients and controls.*

Results. *A higher number of SS patients exhibited elevated levels of lactoferrin as compared to controls (86% vs. 20%, respectively). No significant difference was observed in the mean level of total iron in the saliva between patients and controls (12.6 µg/100 ml vs. 11.1 µg/100 ml, respectively). However, the total iron binding capacity of lactoferrin was significantly lower among SS patients than healthy controls (38.2 µg/100 ml vs. 61.8 µg/100 ml, respectively), p = 0.019.*

Conclusion. *The overall results of this study suggest a possible impairment of the iron binding capacity of saliva in SS patients. Such impairment may contribute to the cellular damage of the salivary glands observed in SS patients.*

Introduction

Sjögren's syndrome (SS) is a chronic salivary gland disorder characterized by lymphocytic infiltration of the salivary and lacrimal glands (1-3). Elevated concentrations of total salivary protein, albumin, cystatin C, cystatin S, lactoferrin, and total IgA have been reported to be associated with reduced salivary secretion in SS (4, 5). Lactoferrin (LF) is an iron binding protein that provides significant protection against the oxidative insults of hydrogen peroxide and UV irradiation (6). Such protection is provided by iron-unsaturated LF, while iron-saturated LF offers no protection against oxidative insults (6).

The purpose of this study was to compare changes in the level of salivary lactoferrin, total iron, iron binding capacity, and saturated/unsaturated iron binding capacity of parotid saliva of SS patients and controls.

Material and methods

Patients

102 Sjögren's syndrome patients (13 males and 89 females) attending the Salivary Dysfunction Clinic at Baylor College of Dentistry were included in this study. The diagnosis of Sjögren's syndrome (SS) was based on the European Community Criteria (7). All patients had dry mouth, dry eyes, at least one positive autoantibody against SS-A, SS-B, rheumatoid factor, or antinuclear antibody and/or a positive minor salivary gland biopsy (7, 8). The majority of patients (97) had primary SS; only 5 patients had secondary SS. Twenty individuals (7 males and 13 females) without SS were selected as controls (HC).

Saliva collection and salivary elelctrophoresis

Stimulated human parotid saliva (HPS) collection, salivary protein determination, and salivary electrophoresis were performed as previously described (9-11). The identity of LF was verified by Western blot analysis using commercial anti-LF antibodies and human milk LF as the control (9).

Iron binding of saliva and salivary lactoferrin

LF was separated into apo-LF and iron saturated LF on a 7.5% non-reducing SDS-PAGE and stained with Coomassi blue, according to Kijlstra *et al.* (12). Ferric nitrate was used as iron chelator. The percentage of apo-LF and iron saturated LF was measured densitometrically using NIH Image 1.5 software (NIH, Bethesda MD). Total salivary iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), and percent saturated iron binding capacity (%SIBC) was determined according to Fischer and

Price (13). The ratios of iron saturated:unsaturated (LF and salivary binding capacity) were also calculated for both patients and controls.

Statistical analyses

The Mann-Whitney U test at $p < 0.05$ was used to compare the flow rate, total salivary protein and salivary iron,TIBC, UIBC, and %SIBC of saliva between patients and controls. Student's t-test at $p < 0.05$ was used to compare the age between SS and HC. Differences in LF on SDS-PAGE between the SS and HC groups were evaluated using a Chi-square test at $p < 0.05$.

Results

Study population

Table I shows the demographics of the study population. SS patients had a significantly lower mean salivary flow rate than the HC (0.35 ± 0.02 ml/min/gland vs. 0.45 ± 0.04 ml/min/gland, respectively), p = 0.0326.

LF in saliva

Figure 1 shows the typical SDS-PAGE profile of saliva under reducing conditions. LF with an apparent molecular weight of 90 kDa was observed in 86% of SS patients and 20% of HC. The identity of LF was verified by Western blot analysis (data not shown). LF was prominent in a significantly higher number of SS than HC, p = 0.0001.

Iron saturated/unsaturated LF

Figure 2 shows the iron binding characteristics of LF under non-reducing conditions and without heating the samples. Apo-LF displayed an apparent molecular weight of 68 kDa. However, in the presence of iron chelate an additional LF band (iron-saturated LF) with an apparent molecular weight of 60 kDa was observed. The identity of apo-LF and iron saturated LF was verified by Western blot analysis (data not shown). Densitometric measurements of the two LF bands on SDS-PAGE showed that apo-LF (Fig. 2, lanes HPS + Fe, arrow A) represented approximately 27% of total salivary LF in SS patients as compared to ~54% in HC. In contrast, iron saturated LF (Fig. 2, lanes HPS + Fe, arrow B) represented ~73% of total salivary LF in SS patients as compared to ~46% in HC. The ratio of saturated: unsaturated LF for SS: HC was 3.1.

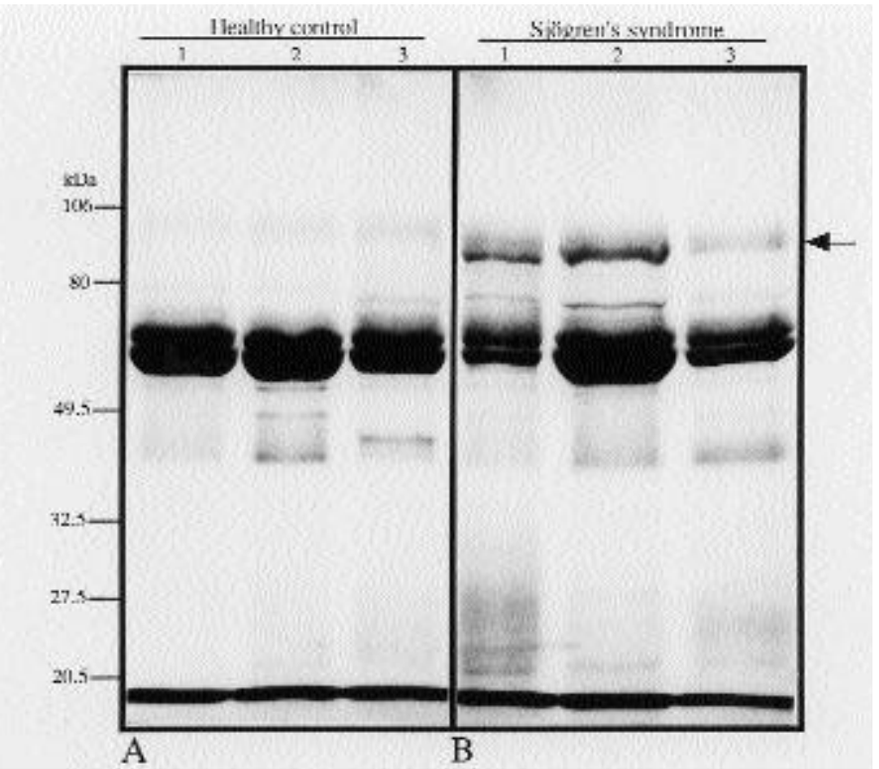

Fig. 1. Electophoretic profile of human parotid saliva (HPS) on 10% SDS-PAGE under reducing conditions stained with Coomassie Brilliant Blue. SS: Sjögren's syndrome patients and HC: healthy controls, 20 µg each. The relative intensity of lactoferrin (**arrow**) is higher among SS than HC.

Iron binding capacity of saliva

The mean total iron in parotid saliva for HC was 11.1 ± 1.1 µg/100 ml and 12.6

Table I. Demographics and salivary parameters of the study population.

	HC			SS			
Variable	N	Median	Mean ± SEM	N	Median	Mean ± SEM	P *
Age (yrs.)	20	58	56.1 ± 3.6	102	60	59.5 ± 1.32	0.319
Salivary flow rate (ml/min/gland)	20	0.43	0.45 ± 0.04	102	0.32	0.35 ± 0.02	0.032
Salivary protein (mg%)	20	137.5	138.0 ± 14.3	102	128	149.5 ± 8.8	0.849

SEM: Standard error of the mean.
* Mann-Whitney U test.

Fig. 2. SDS-PAGE (7.5%) profile of HPS (30 µg) under non-reducing conditions in the presence and absence of iron (+Fe), stained with Coomassie Brilliant Blue. 3 mg human milk lactoferrin (LF) was used as control. Un-saturated lactoferrin; apo-LF (**arrow A**) and iron saturated LF (**arrow B**).

± 1.8 µg/100 ml for SS. A Mann Whitney U test showed no significant differences in total salivary iron between SS and HC, p = 0.716. However, the total iron binding capacity (TIBC) of saliva was 61.8 ± 6.4 µg/100 ml for HC and 38.2 ± 3.6 µg/100 ml for SS patients. The Mann Whitney U test revealed significant differences in TIBC between SS and HC, p = 0.019 (Table II).

The mean unsaturated iron binding capacity (UIBC) of saliva was 50.3 ± 6.5 µg/100 ml for HC and 25.5 ± 3.6 µg/100 ml for SS patients. The Mann Whitney U test showed significant differences in UIBC between HC and SS subjects (p = 0.013, Table II). The mean percent saturated iron binding capacity (%SIBC) of parotid saliva was 21.6 ± 4.0% for HC and 34.6 ± 4.1% for SS patients. The Mann Whitney U test showed significant differences in %SIBC between HC and SS (p = 0.045, Table II). The ratio of SIBC: UIBC for SS: HC was 3.2.

The calculated ratio of saturated: unsaturated LF to the ratio of saturated: unsaturated iron binding capacity of saliva was 2.07 for SS patients and 2.1 for HC.

Discussion

In mammalian cells, hydrogen peroxide may be reduced by ferrous iron to form hydroxyl radicals that are extremely damaging to the cells (6, 14). Lactoferrin is a potent iron binding protein which plays a critical role in intracellular protection against oxidative insults (6).

In this study we compared the status of lactoferrin, total iron, total iron binding capacity, and the total saturated/unsaturated iron binding capacity of human parotid saliva between SS patients and healthy controls. Our result confirms previous studies in that higher levels of LF were observed among SS patients than controls (4, 5, 11). However, our results also indicated that the saliva of SS patients contains proportionally lower levels of unsaturated LF (apolactoferrin) than healthy controls (27% vs. 54%, respectively) (Fig. 2). In addition, the saliva of SS patients showed significantly lower total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) than HC (p = 0.019 and p = 0.013, respectively) (Table II). In addition, a proportionally higher saturated iron binding capacity (SIBC) was observed among SS patients than HC. Interestingly, the ratio of saturated to unsaturated LF in SS versus HC was almost identical to the ratio of saturated to unsaturated iron binding capacity of saliva (3.1 versus 3.2). This suggests that LF is the major iron binding protein in saliva, which is supported by the equal proportional ratio of saturated: unsaturated LF to the ratio of saturated: unsaturated iron binding capacity of saliva for both patients and controls (2.07 versus 2.1). Therefore, the lower level of UIBC along with the higher %SIBC of saliva in patients may suggest a potential role for LF and/or iron in the pathogenesis of SS. These results imply a potential role for oxidative injury in the pathogenesis of SS. This is in agreement

Table II. The status of salivary iron in healthy control (HC) and Sjögren's syndrome (SS) subjects.

	HC			SS		
Salivary iron	N	Median	Mean ± SEM	Median	Mean ± SEM	P *
Free iron (µg/100 ml)	22	11.8	11.1 ± 1.1	12	12.6 ± 1.8	0.716
TIBC (µg/100 ml)	22	72	61.8 ± 6.4	34	38.2 ± 3.6	0.019
UIBC (µg/100ml)	22	57.7	50.3 ± 6.5	21.7	25.5 ± 3.6	0.013
%SIBC	22	16.4	21.6 ± 4.0	38.2	34.6 ± 4.1	0.045

SEM: Standard error of the mean; TIBC: Total iron binding capacity; UIBC: Unsaturated iron binding capacity; %SIBC: Percent saturated iron binding capacity.
* Mann-Whitney U test

with previous studies which have suggested that unsaturated apo-LF, but not iron-saturated LF, is a powerful inhibitor of oxidative damage (6, 14, 15).

This is the first quantitative demonstration of apo- and saturated lactoferrin in human saliva. It is also the first study that demonstrates a proportional increase in the levels of saturated lactoferrin and salivary iron binding capacity among SS patients. The overall result of this study provides a potential mechanism for acinar injury in SS.

References

1. Al-HASHIMI I: The management of Sjögren's syndrome in dental practice. *J Am Dent Assoc* 2001; 132: 1409-17.
2. TALAL N: What is Sjögren's syndrome and why is it important? *J Rheumatol* 2000; 61S: 1-3.
3. Al-HASHIMI I, WRIGHT JM, COOLEY CA, NUNN ME: Reproducibility of biopsy grade in Sjögren's syndrome. *J Oral Path Med* 2001; 30: 408-12.
4. VAN DER REIJDEN WA, VAN DER KWAAK JS, VEERMAN ECI, NIEUW AMERONGEN AV: Analysis of the concentration and output of whole salivary constituents in patients with Sjögren's syndrome. *Eur J Oral Sci* 1996; 104: 335-40.
5. KONTTINEN YT, KULOMAA M, MALMSTROM M, KILPI A, REITAMO S: Lactoferrin in Sjögren's syndrome. *Arthritis Rheum* 1984; 27: 462-7.
6. SHIMMURA S, SUEMATSU M, SHIMOYAMA M, TSUBOTA K, OGUCHI Y, ISHIMURA Y: Sub-threshold UV radiation-induced peroxide formation in cultured corneal epithelial cells:the protective effects of lactoferrin. *Exp Eye Res* 1996; 63: 519-26.
7. VITALI C, BOMBARDIERI S, MOUTSOPOULOS H *et al.*: Preliminary criteria for the classification of Sjögren's syndrome. *Arthritis Rheum* 1993; 36: 340-7.
8. GREENSPAN JS, DANIELS TE, TALAL N, SYLVESTER RA: The histopathology of Sjögren's syndrome in labial salivary gland biopsies. *Oral Surg Oral Med Oral Path* 1974; 37: 217-29.
9. ZIPP MM, YASBIN L, Al-HASHIMI I: The effect of parotid salivary flow rate of the levels of salivary antimicrobial proteins in patients with Sjögren's syndrome. *Quintessence Int* 1999; 30: 700-5.
10. LUNDY FT, AL-HASHIMI I, REESE TD, LAMEY PJ: Evaluation of major parotid glycoprotein in patients with burning mouth syndrome. *Oral Surg Oral Med Oral Path Oral radiol Endod* 1997; 83: 252-8.
11. Al-HASHIMI I, HAGHIGHAT N: Salivary electrophoresis in the diagnosis of Sjögren's syndrome. *Oral Surg Oral Med Oral Path Oral Radiol Endod* 1998; 85: 542-7.
12. KIJLSTRA A, KUIZENGA AB, VAN DER VELDE M, VAN HAERINGEN NJ: Gel electrophoresis of human tears reveals various forms of tear lactoferrin. *Current Eye Research* 1989; 8: 581-8.
13. FISCHER D, PRICE D: A simple serum iron method using the new sensitive chromogen tripyridyl-s-triazine. *Clin Chem* 1964;10:21-31.
14. GUTTERIDGE JMC, PATERSON SK, SEGAL AW, HALLIWELL B: Inhibition of lipid peroxidation by the iron-binding lactoferrin. *Biochem J* 1981; 199: 259-61.
15. WINTERBOURN CC: Lactoferrin-catalysed hydroxyl radical production. *Biochem J* 1983; 210: 15-19.