Salivary and serum hyaluronic acid concentrations in patients with Sjögren’s syndrome

Moshe Tishler, Ilana Yaron, Idit Shirazi, Michael Yaron

Abstract

**Objective**—To evaluate salivary hyaluronic acid (HA) concentration in patients with primary Sjögren’s syndrome (SS).

**Methods**—Salivary and serum HA concentrations were evaluated using a radioimmunoassay. Thirty nine patients with SS served as the study group and their results were compared with 19 patients having clinical symptoms and signs of dry mouth and with 10 normal controls.

**Results**—Salivary HA concentrations were significantly increased (p < 0.05) in the 39 patients with SS compared with the 19 patients with dry mouth and the 10 normal controls (240.7 (38.5) v 99.8 (14.6) and 91.3 (7.9) ng/ml, respectively) (mean (SEM)). No significant differences were noted in the serum HA concentrations between the three groups (42 (3.9) v 36.3 (4.3) ng/ml, respectively) (mean (SEM)). No correlation could be found between salivary HA concentrations and the focus score of lip biopsies, nor between salivary HA concentrations and erythrocyte sedimentation rate or other serological tests.

**Conclusion**—Increased salivary HA concentrations can serve as a marker of local inflammation and may be of value in the diagnosis of SS.

(Saliva and serum hyaluronic acid concentrations in patients with primary Sjögren’s syndrome) Moshe Tishler, Ilana Yaron, Idit Shirazi, Michael Yaron

Sjögren’s syndrome (SS) is an autoimmune disorder affecting mainly the exocrine glands, thus causing dryness of the eyes and mouth.1 Salivary gland involvement in SS has been evaluated traditionally by salivary flow rate, scintigraphy, and lip biopsy.2 The typical biopsy findings demonstrate a focal periductal infiltrate composed of mononuclear cells, predominantly CD+ T lymphocytes.3 Furthermore, recent studies have shown that epithelial salivary gland cells produce large amounts of mRNA for various proinflammatory cytokines including interleukin (IL)1, IL6, and IL10.4 The concept that salivary analysis could be used as a diagnostic tool is attractive because it might spare biopsy procedure in some patients. Recently, we and others have shown that increased concentrations of salivary eicosanoids as well as IL6 may serve as a marker of local inflammation in SS.5,6 Hyaluronic acid (HA), which is an unbranched polysaccharide, has been found to be an indicator of connective tissue turnover. Increased serum concentrations of HA has been found in lung and liver diseases as well as in rheumatoid arthritis (RA).6,7 The purpose of our study was to investigate the possibility that increased HA concentrations might be found in the saliva of patients with SS as a consequence of a local inflammatory process.

**Methods**

**STUDY GROUPS**

Thirty nine consecutive patients with primary SS followed up at the SS Clinic, Department of Rheumatology, Tel Aviv Medical Centre, were enrolled in the study. All patients were diagnosed as having SS according to the newly proposed criteria of the EC Study Group.8 All patients had a positive lip biopsy specimen with a focus score > 1, and biopsy specimens were read blindly by a pathologist without knowing details on patients’ clinical data. None of the participating subjects had taken NSAIDs or immunosuppressive treatment for a month before saliva collection, nor had any apparent oral infection. Results from these patients were compared with data of 19 patients referred to the clinic with clinical symptoms and objective signs of dry mouth who did not meet the EC criteria for SS and had a negative lip biopsy specimen (“dry mouth” group). In 10 patients of this group the cause was found to be drug induced, one patient had sarcoidosis, and in eight patients no apparent disorder was detected. A group of 10 normal healthy people served as a control group. Table 1 shows the clinical and demographic data of the study groups. No differences were noted between the study groups as regards to age and salivary flow rates.

**Table 1 Clinical and serological data of the study groups**

<table>
<thead>
<tr>
<th></th>
<th>SS group (n=36)</th>
<th>Dry mouth group (n=19)</th>
<th>Normal controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y) (SD)</td>
<td>58.3 (11.2)</td>
<td>61.3 (9.6)</td>
<td>62.1 (12.3)</td>
</tr>
<tr>
<td>Disease duration (y) (SD)</td>
<td>7.2 (2.2)</td>
<td>6.1 (3.1)</td>
<td>—</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>2/34</td>
<td>2/17</td>
<td>2/8</td>
</tr>
<tr>
<td>RF &gt; 1:160 (%)</td>
<td>70</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>ANF &gt; 1:80 (%)</td>
<td>66</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Anti-Ro antibodies (%)</td>
<td>55</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-La antibodies (%)</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Extraglandular complications (%)</td>
<td>8</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Salivary flow rate (ml/min) (SD) (non-stimulated)</td>
<td>0.07 (0.02)</td>
<td>0.1 (0.02)</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND = Not done.
Hyaluronic acid concentrations in patients with Sjögren’s syndrome

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S. person’s flow rate. No significant di-

viation HA was calculated according to the

No change of results has been found when sali-

ary HA concentrations of blood and saliva were

determined using a radiometric assay (Kabi

Pharmacia, Uppsala, Sweden). The test is

based on the use of specific hyaluronic acid

binding proteins (HABP) isolated from bovine

cartilage. The intra and interassay coefficients

of variation are 5.2% and 6.4%, respectively

and the lower limit of detection is 10 µg/l.

STATISTICAL ANALYSIS

Data for the different groups were initially

evaluated using analysis of variance. The

significance of differences was analysed by

Wilcoxon signed rank tests. Significance was
determined by p value of less than 0.05

Results

Figure 1 gives the results of HA concentrations

in saliva and serum.

The mean (SEM) concentrations of salivary

HA were significantly higher in SS patients

(240.7 (38.5) ng/ml) than in patients with dry

mouth (99.8 (14.6) ng/ml) and in normal

healthy controls (91.3 (7.9) ng/ml) (p < 0.05).

No correlation was found between salivary

HA concentrations and erythrocyte sedimenta-

tion rate, anti-Ro antibodies or antinuclear fac-

tor. Furthermore, salivary HA concentrations

were not different between SS patients with

focus score of 1 and 2.

Discussion

The salivary glands are one of several major

target organs of inflammation in primary SS,

resulting in dry mouth (xerostomia) associated

with reduction of secretory function. As

xerostomia is non-specific and can result from

a variety of causes in up to 20% of the general

population, it is important to find an objective

laboratory test to evaluate the diagnosis of SS

in these patients. The presence of focal

lymphocytic infiltration in the salivary glands

of patients with SS led us to look for inflamma-

tory markers in the saliva of these patients. In

earlier studies, we and others have shown that

increased salivary concentrations of eicosan-

oids (PGE2, TXB2) and of IL6 might be a

good marker to differentiate SS patients from

those suffering from dry mouth without an

accompanying disease. HA was chosen for

this study because it has been shown to be a

marker for inflammation in various disorders.

HA is an unbranched high molecular weight

polysaccharide synthesised by connective tis-

sue cells and is widely distributed in the body.

Raised concentrations of HA have been shown

in bronchoalveolar lavage of patients with

sarcoidosis and idiopathic pulmonary fibrosis.

Similarly, increased serum HA concentrations

have been reported in various liver diseases

where they serve as a marker for progressive

liver damage, especially in primary biliary

cirrhosis. Although increased serum HA is not

specific for the diagnosis of RA as it could be
detected in other forms of arthritis, it has

been shown to be a useful marker of disease

activity.

A number of immune regulatory functions

are associated with HA, notably its effects on T

cells and in turn its production rate is

stimulated by proinflammatory cytokines, such

as IL1, IL6, and tumour necrosis factor. We

speculated that increased HA concentrations

could be found in the saliva of SS patients as a

consequence of local production. Therefore

the finding of a significant increase in salivary

HA in patients with primary SS as compared

with a dry mouth group and normal controls is

not surprising. To the best of our knowledge,

these findings are unique and have not been

described before. Indeed, our finding of

increased HA concentrations in the saliva of

patients with SS is in accordance with recent

studies that showed large amounts of mRNA

for IL1, IL6, and IL10 in the epithelial salivary

glands of these patients.

Increased salivary HA concentrations in

patients with SS could be explained by two

mechanisms: (a) local production, (b) a loss

of structural barriers in the gland parenchyma,

allowing HA to enter saliva from the serum.

However, the latter seems unlikely as studies of

saliva composition in SS have shown that the

epithelial barriers remain intact. Furthermore,
in our study serum HA in all three groups stud-

ied were not statistically different, thus support-
ing the idea that increased salivary HA concen-

trations are a consequence of local production.

We think that the “leak” of HA to the saliva

rather than into the serum as well is probably

because the total amount of HA produced by

Figure 1 Salivary and serum hyaluronic acid concentrations in the study groups (mean (SEM)).
the salivary gland is small. This is in contrast with other diseases, such as RA, in which there are multiple joint effusions causing large quantities of HA to be released from the synovium into the serum. The fact that increased HA salivary concentrations were found in SS patients and not in patients with dry mouth despite similar flow rates, rules out the possibility that small salivary volume contributed to the increased concentrations. As evaluation of salivary gland involvement in SS is complicated and usually requires biopsy, we suggest that salivary HA may be added to the list of markers that can be easily used in supporting the diagnosis of SS in patients presenting with dry mouth symptoms. Of course, it should be borne in mind that it cannot be used as a marker of salivary disease severity or activity as has been shown in salivary IL6.

Although HA is thought to be a good marker for collagen turnover and also of inflammation, we have no explanation for the fact that its concentrations do not correlate with either disease activity or severity. There is no doubt that a bigger control group with a wide age range is needed to calculate the sensitivity, and comparison with other autoimmune diseases could for SS clarify the specificity of this novel marker.

12 Butler DM, Vitti GF, Leizer T, Hamilton JA. Stimulation of the hyaluronic acid levels of human synovial fibroblasts by recombinant human tumor necrosis factor α, tumor necro-
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