Salivary CXCL13 in relation to scintigraphy in early detection of secondary Sjogren’s syndrome
Salwa G. Moussa, Hanan E. El-Hefnawy, Heba F. El-Shishtawy, Dalia M.E. El Mikkawy, Mennatallah H. Shalaby

Background
Sjogren’s syndrome (SS) is a systemic autoimmune disease in which immune cells attack and destroy the exocrine glands. CXCL13 directs B-cell chemotaxis and is elevated in several autoimmune diseases.

Objective
To assess the role of salivary CXCL13 level as a screening tool in early detection of secondary SS patients.

Patients and methods
Salivary CXCL13 levels using ELISA technique, Schirmer paper test and/or Lissamine green staining, and quantitative salivary scintigraphy excretion fraction were measured in 45 selected patients with primitive connective tissue disease (rheumatoid arthritis, systemic lupus erythematosus, or systemic sclerosis) and according to the American-European Consensus Group criteria, they were classified to three equal groups: group I were having SS; group II were having dryness manifestations but not completing the criteria for SS diagnosis (suspected SS); group III were having no SS, and 15 age-matched and sex-matched apparently healthy controls.

Results
A significantly higher salivary CXCL13 level on comparing SS patients to suspected, non-SS groups and controls (p < 0.001). Salivary CXCL13 had a significant negative correlation with scintigraphy (p < 0.01), a significant positive correlation with eye dryness signs (p < 0.01), cutoff value of CXCL13 to diagnose SS was more than 40 pg/ml and a cutoff value of salivary scintigraphy excretion fraction to diagnose SS was less than 33.1%.

Conclusion
Salivary CXCL13 is a sensitive biomarker for early detection of secondary SS.

Keywords:
CXCL13, salivary, scintigraphy, Sjogren’s syndrome

Introduction
Sjogren’s syndrome (SS) is a systemic autoimmune disease in which immune cells attack and destroy the exocrine glands that produce tears and saliva [1].

B cells have been implicated in the pathogenesis of SS, as they secrete inflammatory cytokines and produce autoantibodies that focus inflammation. Significantly, B cells also influence the differentiation of T-helper cells, specifically Th17 and T regulatory subsets, which are implicated in the modulation of autoimmune disease [2].

It has been suggested that chronic inflammation of exocrine glands depends on chemokines, which control the selective traffic and tissue homing of inflammatory cells [3].

CXCL13 directs B-cell chemotaxis and is elevated in several autoimmune diseases. A study demonstrates that in humans, CXCL13 is elevated in the serum and saliva, and an elevated salivary CXCL13 level distinguishes patients with xerostomia [4,5]. These data suggest a role for CXCL13 as a valuable biomarker in SS, as 74% of patients with SS displayed elevated CXCL13 in the sera, saliva, or both [5]. Thus, CXCL13 may be pathogenically involved in SS and may serve as a new marker and as a potential therapeutic target.

Salivary scintigraphy is a noninvasive method to evaluate the function of salivary glands by addressing the uptake and secretion of a radioactive-labelled substance (sodium pertechnate of 99mTc). Additionally, an abnormal salivary gland scintigraphy result is accepted by the

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American-European Consensus Group as a criterion for
the diagnosis of SS [6].

Patients and methods
The present study is a case–control one that included
45 patients having either rheumatoid arthritis, systemic
lupus erythematosus, or systemic sclerosis. They were
selected from inpatients and outpatients in the Ain
Shams University Hospitals. Fifteen age-matched and
sex-matched apparently healthy individuals were also
included in the study and served as a control group.
Written consent was obtained from all patients and
controls after a full explanation of the study was
provided. Ethics committee approval was obtained
for the study.

The selected patients were divided into three equal
groups as follows: patients with SS according to the
American-European Consensus Group criteria [7],
patients suspicious to have SS (not fulfilling the
criteria), and patients with no manifestations of SS.

Exclusion criteria
Past head and neck radiation treatment, use of
anticholinergic drugs, facial paralysis, diabetes
mellitus, multiple sclerosis, graft-versus-host disease,
sarcoidosis, hepatitis C virus, and pregnant ladies.

All patients were subjected to laboratory investigations
such as salivary CXCL13 levels using ELISA
technique, complete blood picture, erythrocyte
sedimentation rate, and C-reactive protein.

For dry eye diagnosis Lissamine green (LG) staining
and/or Schirmer paper test were done (Figs 1 and 2).

For dry mouth diagnosis quantitative salivary gland
scintigraphy to evaluate the function of salivary glands
by addressing the uptake and secretion of a radioactive-
labelled substance (sodium pertechnate of $^{99m}$Tc) and
then calculate the excretion fraction (EF) (Fig. 3).

Statistical presentation and analysis was carried out by
IBM SPSS statistics (version 17.0, 2009; IBM Corp.,
Chicago, USA) used for data analysis. The mean and
SD, the medians, Student $t$-test, $\chi^2$, Kruskal–Wallis test,
and analysis of variance tests were used to summarize
the characteristics of the samples. Categorical variables were
summarized with frequencies and percentages.
Spearman’s correlation coefficients were established
with continual values of salivary CXCL13, salivary
gland scintigraphy EF, and eye dryness signs,
depending on the distribution of the variables.
Receiver operating characteristic (ROC) curves were
calculated to establish sensitivity, specificity for
CXCL13 salivary level, and scintigraphy EF. A value
of $P$ less than 0.05 was considered significant.

Figure 1

Lissamine green temporal stain (moderate degree) in right. Eye of a
rheumatoid arthritis patient with secondary Sjogren’s syndrome.

Figure 2

Schirmer paper test showing positive results in both patients (<5 mm
in 5 min).

Figure 3

Quantitative salivary gland scintigraphy of an Sjogren’s syndrome
patient before applying lemon juice at 10–12 min postinjection, after
applying lemon juice at 18–20 min postinjection. Region of interest
(ROIs) used for quantification are depicted on the left scintigram.
Results
This study included 45 patients with primitive connective tissue disease (rheumatoid arthritis, systemic lupus erythematosus, or systemic sclerosis), classified according to the American-European Consensus Group criteria for SS diagnosis into three equal subgroups: group I, group II, and group III.

Group I included 15 patients with SS, 15 (100%) women and zero (0.0%) men; their ages ranged from 30.0 to 58.0 years with a median of 41 years. Their dryness manifestations duration ranged from 0.58 to 10.0 years, with a median of 2 years.

The salivary CXCL13 level ranged from 44 to 365 pg/ml (mean±SD 147.87±97.74).

Group II included 15 patients with suspected SS (having dryness manifestations but not completing criteria of SS diagnosis), 14 (93.3%) women and one (6.7%) man; their ages ranged from 15 to 60 years with a median of 50 years. Their dryness manifestations duration ranged from 0.08 to 15 years, with a median of 4 years.

The salivary CXCL13 level ranged from 11 to 142 pg/ml (mean±SD 46.9±35.4).

Group III included 15 patients with no SS (not suspected nor having SS), 14 women (93.3%) and one (6.7%) man; their ages ranged from 17 to 58 years with a median of 41 years.

The salivary CXCL13 level ranged from 10 to 34 pg/ml (mean±SD 23.57±8.63).

Schirmer paper test and/or LG stain were positive in 15 (100%) patients.

Group III included 15 patients with no SS (not suspected nor having SS), 14 women (93.3%) and one (6.7%) man; their ages ranged from 17 to 58 years with a median of 41 years.

The salivary CXCL13 level ranged from 10 to 34 pg/ml (mean±SD 23.57±8.63).

Schirmer paper test and/or LG stain were positive in nine (60.0%) patients.

The clinical, laboratory, and scintigraphy data of the patients are shown in Tables 1–3.

There was a highly significant increase in salivary CXCL13 in SS patients versus the suspected SS, non-SS, and control groups (P<0.001). There was a significant increase in salivary CXCL13 in suspected SS group versus the control group (P=0.031). There was a statistically significant positive relation between

<table>
<thead>
<tr>
<th>Ocular dryness manifestations</th>
<th>Groups [n (%)]</th>
<th>χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SS group</td>
<td>Suspected group</td>
<td>No SS group</td>
</tr>
<tr>
<td>LG or SP test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0 (0.0)</td>
<td>6 (40.0)</td>
<td>15 (100.0)</td>
</tr>
<tr>
<td>Positive</td>
<td>15 (100.0)</td>
<td>9 (60.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Dry for 3 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>1 (6.67)</td>
<td>8 (53.33)</td>
<td>15 (100.0)</td>
</tr>
<tr>
<td>Positive</td>
<td>14 (93.33)</td>
<td>7 (46.67)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Sand sensation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>6 (40.0)</td>
<td>8 (53.33)</td>
<td>15 (100.0)</td>
</tr>
<tr>
<td>Positive</td>
<td>9 (60.0)</td>
<td>7 (46.67)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Artificial tears three times/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>9 (60.0)</td>
<td>6 (40.0)</td>
<td>15 (100.0)</td>
</tr>
<tr>
<td>Positive</td>
<td>6 (40.0)</td>
<td>9 (60.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total of positive eye manifestations</td>
<td>15 (100%)</td>
<td>13 (86.7)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

It shows a highly statistically significant difference between patient groups as regards dryness manifestations (SS > suspected SS > non-SS). LG, Lissamine green; SP, Schirmer paper test; SS, Sjogren’s syndrome. *Means significant difference.
salivary CXCL13 levels and positive eye staining and Schirmer paper test.

There was a significant negative correlation between salivary CXCL13 levels and salivary gland scintigraphy EF ($r = 0.65$, $P < 0.001$) (Table 4). There was insignificant relation between salivary CXCL13 levels and either sex, age, or duration of SS ($P > 0.05$). Using salivary CXCL13 levels above 40 pg/ml as a cutoff point, the ROC curve analysis showed a sensitivity of 100% and a specificity of 60%, with an area under the curve of 0.871 (95% confidence interval: 0.698–0.965) (Fig. 4).

There was a statistically significant difference between salivary gland scintigraphy EF of SS group and other groups ($P < 0.001$). There was no significant correlation between salivary gland scintigraphy and duration of SS ($r = 0.292$, $P > 0.05$). The salivary gland scintigraphy EF cutoff value was less than 33.1% with a sensitivity of 100% and a specificity of 86.7% was used in the diagnosis of oral dryness in SS (Fig. 5).

**Table 2** Comparison between patients and control groups as regards salivary CXCL13 levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>CXCL13 saliva (pg/ml)</th>
<th>Kruskal–Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Median</td>
</tr>
<tr>
<td>SS group</td>
<td>44–365</td>
<td>134.00</td>
</tr>
<tr>
<td>Suspected SS group</td>
<td>11–142</td>
<td>34.00</td>
</tr>
<tr>
<td>No SS group</td>
<td>10–34</td>
<td>24.00</td>
</tr>
<tr>
<td>Control group</td>
<td>0.1–39.1</td>
<td>27.00</td>
</tr>
</tbody>
</table>

**Table 3** Comparison between patients and control group as regards salivary gland scintigraphy (excretion fraction) of the right parotid gland

<table>
<thead>
<tr>
<th>Groups</th>
<th>Salivary scintigraphy of right parotid gland (EF%)</th>
<th>$F$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean±SD</td>
<td></td>
</tr>
<tr>
<td>SS group</td>
<td>7.6–49</td>
<td>22.58±10.81</td>
<td>11.11</td>
</tr>
<tr>
<td>Suspected group</td>
<td>25.2–47</td>
<td>34.05±6.84</td>
<td></td>
</tr>
<tr>
<td>No SS group</td>
<td>25.9–55.6</td>
<td>39.39±9.29</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>26.7–59.4</td>
<td>38.27±8.28</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4** Correlation between levels of salivary CXCL13 and salivary glands scintigraphy excretion fraction

<table>
<thead>
<tr>
<th>Salivary gland scintigraphy (EF%)</th>
<th>CXCL13 saliva</th>
<th>$r$</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary scintigraphy right parotid</td>
<td>−0.610</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Salivary scintigraphy left parotid</td>
<td>−0.629</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Salivary scintigraphy right submandibular</td>
<td>−0.679</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>Salivary scintigraphy left submandibular</td>
<td>−0.671</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Sjogren’s syndrome is a systemic autoimmune disease in which immune cells attack and destroy the exocrine glands that produce tears and saliva [1]. The discovery of novel markers and diagnostic methods is crucial for the diagnosis and management of SS, as a disease stage criteria are lacking, and there is no standardized way to monitor SS progression [8].

In our study comparison between patients and controls regarding salivary CXCL13 revealed a highly significant increase ($P < 0.001$) in salivary CXCL13 in patients compared with controls (134 vs. 27 pg/ml), respectively, suggesting a pathogenic link between CXCL13 and SS, this is in agreement with Kramer et al. [8], a statistically significant difference between SS group versus suspected SS, also between SS
group versus NSS group and SS group versus control group, and suspected SS group versus control group ($P<0.001$). The difference was not statistically significant between suspected group versus non-SS group, and non-SS versus the control ($P>0.05$). So CXCL13 salivary levels increased markedly in patients with SS and to a little extent in suspected patients.

As B cells have been implicated in the pathogenesis of SS [2], and CXCL13 directs B-cell chemotaxis [4], this might explain the rising of salivary CXCL13 in SS patients.

The observation of Lee et al. [9] that the expression levels of CXCL13 within the lymphocytic infiltrates of SS patients was associated with several laboratory features of the disease, lymphadenopathy, and the extent of clinical disease activity recommends CXCL13 as a useful marker for predicting SS disease activity and prognosis.

The cutoff value of salivary CXCL13 to diagnose SS was 40 pg/ml, with a sensitivity of 100% and specificity of 60%, while in Kramer et al. [8], the cutoff value was 100.3 pg/ml. The difference in the results might be due to age difference, patients’ selection and numbers, and different methods of assessment.

Salivary gland scintigraphy using $^{99m}$Tc-pertechnetate is the most commonly used radioactive pharmaceutical agent, which roughly correlates with salivary gland biopsy findings, and it is a direct test of secretory function [10]. In this test, the isotope is concentrated...
and excreted by the salivary glands, which allows demonstration of uptake in the salivary glands.

The EF parameter would be most useful to track subtle changes in the salivary gland function, providing a marker for follow-up with time as stated by Loutfi et al. [11].

In our study on comparing EF of salivary glands of the four groups, the SS group was significantly less than those in the other non-SS groups (P<0.001), and this is in accordance with Adams et al. [12]. The level of large salivary glands EF of up to 33.1% was determined in our study to identify salivary gland dysfunction with a sensitivity of 100% and specificity of 86.67%, while in Klutmann et al. [13] the cutoff value for parotid gland EF was less than 28.3%, and in Schizukuishi et al. [14] it was less than 20.7% in the submandibular gland.

There was a negative correlation between salivary CXCL13 levels and salivary glands EF (P<0.001), this is in agreement with Nishikawa et al. [15].

Strength of the study: it is the first study to detect scintigraphy in SS in an Egyptian population.

Weak aspects: the small number of patients is a weak point. Future study is recommended on a larger scale of patients.

Conclusion

Our results recommend the use of salivary CXCL13 as a noninvasive screening tool for the detection of SS.

Larger scale future studies should be done in order to examine the diagnostic as well as the prognostic role of salivary CXCL13 for early detection of SS and for the evaluation of its value in determining the prognosis of this autoimmune disorder.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References