Salivary CXCL13 in relation to scintigraphy in early detection of secondary Sjogren's syndrome

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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.

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 ^{99m}Tc). Additionally, an abnormal salivary gland scintigraphy result is accepted by the

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, χ^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 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.

Figure 1

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

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).

The salivary gland scintigraphy EF (%) of the right (Rt) parotid gland (mean±SD 22.58±10.8), left (Lt) parotid gland (mean±SD 24.58±7.12), Rt submandibular gland (mean±SD 30.39±8.85), and Lt submandibular gland (mean±SD 20.58±7.55).

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

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).

The salivary gland scintigraphy EF (%) of the Rt parotid gland (mean±SD 34.05±6.84), Lt parotid gland (mean±SD 35.17±8.47), Rt submandibular gland (mean±SD 19.31±8.68), and Lt submandibular gland (mean±SD 33.59±9.73).

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

The salivary gland scintigraphy EF (%) of the Rt parotid gland (mean±SD 39.39±9.3), Lt parotid gland (mean±SD 37.11±6.4), Rt submandibular gland (mean±SD 34.13±5.59), and Lt submandibular gland (mean±SD 35.40±6.09).

Schirmer paper test and/or LG stain were positive in zero (0.0%) patient.

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 1 Frequencies of ocular dryness manifestations among patient groups

Ocular dryness manifestations	Groups [n (%)]				χ^2	P-value
	SS group	Suspected group	No SS group	Total		
LG or SP test						
Negative	0 (0.00)	6 (40.00)	15 (100.00)	21 (46.67)	30.536	<0.001*
Positive	15 (100.00)	9 (60.00)	0 (0.00)	24 (53.33)		
Dry for 3 months						
Negative	1 (6.67)	8 (53.33)	15 (100.00)	24 (53.33)	26.250	<0.001*
Positive	14 (93.33)	7 (46.67)	0 (0.00)	21 (46.67)		
Sand sensation						
Negative	6 (40.00)	8 (53.33)	15 (100.00)	29 (64.44)	12.996	0.002*
Positive	9 (60.00)	7 (46.67)	0 (0.00)	16 (35.56)		
Artificial tears three times/day						
Negative	9 (60.00)	6 (40.00)	15 (100.00)	30 (66.67)	12.600	0.002*
Positive	6 (40.00)	9 (60.00)	0 (0.00)	15 (33.33)		
Total of positive eye manifestations	15 (100%)	13 (86.7)	0 (0.00)	28 (62.2)	37.6	<0.001*

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.

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

Groups	CXCL13 saliva (pg/ml)			Kruskal–Wallis test	
	Range	Median	IQR	H	P-value
SS group	44–365	134.00	178.00	32.27	<0.001*
Suspected SS group	11–142	34.00	44.00		
No SS group	10–34	24.00	17.50		
Control group	0.1–39.1	27.00	24.60		

Mann–Whitney test					
SS and suspected SS	SS and non-SS	SS and control	Suspected SS and non-SS	Suspected SS and control	Non-SS and control
0.001*	<0.001*	<0.001*	0.056	0.031*	0.95

It shows a statistically significant difference between SS versus suspected SS, SS versus non-SS, SS versus control, and suspected SS versus control. SS, Sjogren's syndrome. *Means significant difference.

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

Groups	Salivary scintigraphy of right parotid gland (EF%)		F	P-value
	Range	Mean±SD		
SS group	7.6–49	22.58±10.81	11.11	<0.001*
Suspected group	25.2–47	34.05±6.84		
No SS group	25.9–55.6	39.39±9.29		
Control group	26.7–59.4	38.27±8.28		

Tukey's test					
SS and suspected	SS and NSS	SS and control	Suspected and NSS	Suspected and control	NSS and control
0.005*	<0.001*	<0.001*	0.37	0.57	0.99

It shows a statistically significant difference between SS versus suspected SS, SS versus non-SS, and SS versus control. EF, excretion fraction; SS, Sjogren's syndrome. *Means significant difference.

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

Salivary gland scintigraphy (EF%)	CXCL13 saliva	
	r	P-value
Salivary scintigraphy right parotid	−0.610	<0.001*
Salivary scintigraphy left parotid	−0.629	<0.001*
Salivary scintigraphy right submandibular	−0.679	<0.001*
Salivary scintigraphy left submandibular	−0.671	<0.001*

It shows a negative statistically significant correlation. EF, excretion fraction. *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).

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
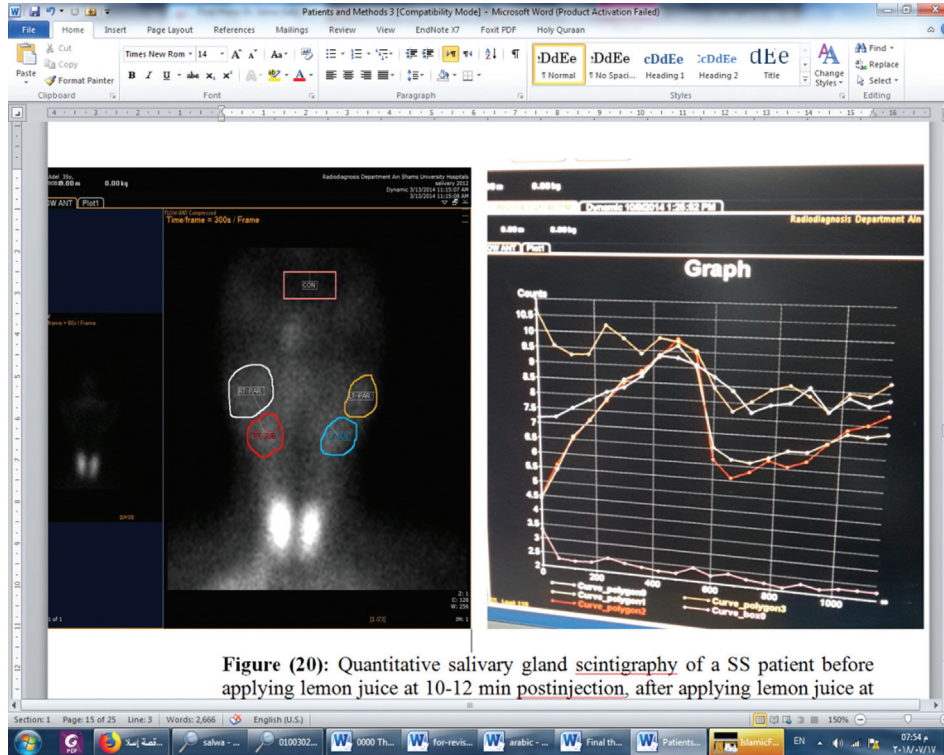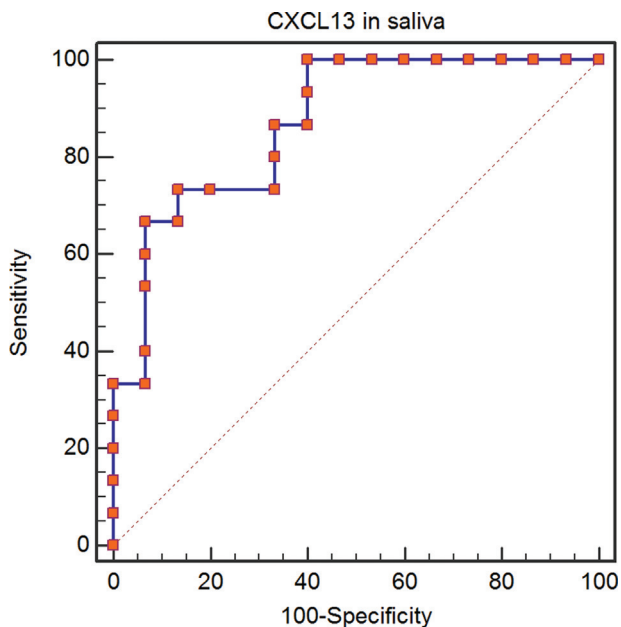
Figure 4

Receiver operating characteristic (ROC) curve for CXCL13 salivary levels

Figure 5

Receiver operating characteristic (ROC) curve for salivary gland scintigraphy.

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.

Conflicts of interest

There are no conflicts of interest.

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