

Quantitative salivary gland scintigraphy can distinguish patients with primary Sjögren's syndrome during the evaluation of sicca symptoms

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Received: 28 December 2006 / Revised: 31 January 2007 / Accepted: 31 January 2007 / Published online: 21 February 2007
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Abstract Abnormal findings on salivary gland scintigraphy (SGS) are part of the classification criteria for Sjögren's syndrome (SS), but SGS is operator dependent and poorly standardised. We studied the use of quantitative data on the uptake, concentration and excretion of the four major salivary glands in the evaluation of sicca patients. During an initial clinical evaluation for sicca symptoms (mean duration, 51 months), 24 subjects were classified as either SS ($n=8$) or isolated sicca (IS; $n=16$). SGS was then performed after i.v. injection of 200 MBq pertechnetat. Digitalised quantitative data on time-to-peak uptake (T_{max}), peak tracer distribution (C%) and stimulated excretion (E%) were calculated from time-activity curves and compared between groups and controls ($n=8$) and correlated to clinical data. Statistical analysis was performed with non-parametric tests. SS patients had longer T_{max} in both parotid glands (18.1 min; $p<0.01$) and both submandibular glands (mean 13.7 min, $p<0.05$); whereas T_{max} in IS patients was similar as in controls in both parotid (10.4 min; $p>0.2$) and submandibular glands (9.4 min; $p>0.4$). C% was significantly lower in the parotid glands of both the SS and the IS group compared to the controls ($p<0.01$). E% was significantly reduced in SS patients (16.3% for parotid and 17.4% for submandibular glands; $p<0.01$); whereas in the IS patients, excretion (32,

2% for parotid and 26, 9% for submandibular glands) was similar from all glands as in the control groups (35, 2% for parotid and 27, 8% for submandibular glands). No correlation was found between these SGS results and age, focus score, erythrocyte sedimentation rate, serum creatinin or immunoglobulin levels. No IS patient progressed to full-blown pSS during the 4 years of follow-up. Quantitative SGS data are useful and objective tools to distinguish patients with SS.

Keywords Quantitative scoring · Salivary scintigraphy · Sicca symptoms

Introduction

Xerostomia is one of the main clinical features of Sjögren's syndrome (SS). Various methods are available to assess salivary gland involvement in SS, which measure different aspects of salivation and do not correspond well to each other. Salivary flow rate reflects both basal saliva production from all glands and the anatomical and functional integrity of the ductal system. Contrast radiography provides data on the morphology and flow velocity of the salivary duct system during invasive cannulation. Salivary gland biopsy can provide definite evidence of the specific inflammation seen in SS but does not give (functional) information for the major glands as it is taken from the minor labial glands [1–5]. These limitations have become more important in the face of emerging evidence that functional antibodies against muscarinic receptors can independently contribute to defective salivation [6, 7]. Salivary gland scintigraphy (SGS) provides a detailed functional evaluation of salivation as it measures the amount and speed of radioisotope uptake and excretion for all four major salivary

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glands separately [8]. Although abnormal SGS findings are included as one of the classification criteria for SS [5], SGS is not a very well standardised procedure, and results are reported in a qualitative way and are operator dependent [9, 10]. The use of computer-assisted time-activity curves allows quantisation of salivary gland radioactivity over defined time periods during SGS [11, 12]. This study evaluated the diagnostic value of quantifying salivary gland uptake and secretion in patients with sicca symptoms.

Materials and methods

Subjects

Patients referred for the evaluation of sicca symptoms were included in this cross-sectional study, and the following data were collected: duration and type of sicca symptoms (as defined in [15]) and results of the Schirmer-1 test, Rose bengal dye uptake, labial biopsy, parotid sialography, unstimulated salivary flow, medication and laboratory investigations (erythrocyte sedimentation rate [ESR], total WBC and lymphocyte count, levels of C-reactive protein, haemoglobin, serum creatinin and liver enzymes, anti-Ro/SS-A antibodies [by ELISA], rheumatoid factor [RF; by nephelometry and Waaler] and anti-ds DNA [by immunofluorescence and ELISA]). Data were extracted from patient records by one investigator with the use of a pre-defined data collection form. Twenty-four patients not fulfilling exclusion criteria for SS [16] were subsequently investigated by SGS. Before SGS and based solely on prior collected clinical data, patients were classified as either SS when fulfilling at least four of the preliminary European criteria or as isolated sicca (IS) when fulfilling less than four of these criteria [15]. The control group consisted of eight euthyroid patients referred for suspected nodular thyroid disease; informed consent for the study was obtained from all participants.

Salivary gland scintigraphy

SGS was performed with a gamma camera (Sophia D57) equipped with a high sensitive collimator (General Purpose). Medication that could influence salivary flow was stopped 2 days before (diuretics in one patient and an anticholinergic in another). The head was immobilized in an anterior position relative to the camera; projection was from the anterior with the camera adjusted above the parotid and submandibular glands and the mouth. Immediately after the intravenous injection of 200 MBq ^{99}Tc -pertechnetat, a dynamic acquiring protocol started, and regions of interest were marked manually while carefully avoiding the inclusion of thyroid tissue; images were then acquired

every 30 s during a period of up to 40 min representing uptake, concentration and spontaneous secretion of tracer. Subsequently, the mouth was then flushed three times with water to empty the oral cavity for radioactivity, and lemon juice was administered after which imaging continued for another 10 min during the stimulated secretion phase. Data were digitally stored and plotted on separate time-activity curves for each salivary gland after manual selection of the glands. All time-activity curves were also visually analysed to ensure that the correct time points were applied in the curve analysis. The computer software calculated the following variables from the time-activity curve for each gland: (1) time to maximal (Tmax), reflecting the time (in minutes) needed to achieve the maximum concentration; (2) C%, reflecting the percent distribution of total uptake in each different gland; and (3) E%, reflecting the stimulated secretion calculated as the percentage of reduction in concentration from the lemon juice stimulation to the lowest value achieved ($E\% = (1 \text{ min}/\text{max}) \times 100\%$).

Statistics

Figures represent mean values or numbers (%), unless indicated otherwise. Dichotomous data were analysed with cross tables using Fisher's exact test, due to small numbers. Analysis of variance was performed for continuous variables. Correlations between continuous variables were analysed by Spearman's rank test. Data were analysed using SPSS v.11.0. Resulting p-values below 0.05 were considered to indicate statistical significance.

Results

Patient characteristics

Before SGS, eight patients were classified for SS diagnosis; whereas the remaining 16 patients were classified as IS. The mean age of patients was 46 years (range 27–63), and 92% were women with no significant differences between SS and IS patients. The mean symptomatic period before SGS was 51 months (range 9–109). Three IS patients had established RA (one patient) or SLE (two patients). Control patients (84% women; $p > 0.2$) were younger than sicca patients (38 years; range 20–67; $p < 0.05$). None of the IS patients progressed to established pSS during the 64 months (range 1–140) of follow-up. Results for routine laboratory findings for SS and IS patients (Table 1) showed significantly higher mean values for ESR, lymphocyte counts and serum IgG levels in the SS group. Four of five SS patients with antibodies against Ro/SSA were also positive for RF, whereas anti-DNA antibodies were not detectable in any patient.

Table 1 Laboratory findings for SS and IS patients

	SS	IS	Total	Reference	<i>p</i> Value
Haemoglobin (g/dl)	12.3	13.3	12.9	11.5–16.0	0.13
Leukocytes ($10^9/l$)	5.9	6.6	6.2	4.0–11.0	0.68
Lymphocytes (%)	48	28	30	20–45	0.03
ESR (mm/h)	36.9	17.1	23.7	<21	0.03
CRP (mg/l)	4.6	4.4	4.5	<5	0.71
Serum creatinin ($\mu\text{mol/l}$)	66.6	72.9	70.6	55–100	0.34
ASAT (U/l)	25.1	25.0	25.1	10–35	0.98
ALAT (U/l)	28.4	40.3	35.9	10–35	0.21
IgG (g/l)	29.9	13.7	19.1		0.008
IgA (g/l)	3.6	2.7	3.0		0.07
IgM (g/l)	2.1	1.5	1.7		0.20
Anti SS-A antibody	5 (63)	0	NA	Negative	0.001
RF-nephelometry	5 (63)	2 (13)	NA	Negative	0.02
RF-Waaler	4 (50)	0	NA	Negative	0.10
Anti-dsDNA antibody	0	0	NA	Negative	–

Figures represent mean values or numbers (%)

CRP C-reactive protein, ASAT aspartate transaminase, ALAT alanine transaminase, Ig Immunoglobulin.

Tc-99m scintigraphy

The Tmax tracer uptake was significantly longer in SS patients only; delayed uptake was seen in both parotid glands (mean 18.1 min; $p < 0.01$) and both submandibular glands (mean 13.7 min, $p < 0.05$; Fig. 1a). Uptake was symmetrically delayed in all glands in SS patients (Fig. 1b). Tracer uptake in IS patients was not delayed compared to the controls in both parotid (10.4 and 8.8 min, respectively; $p > 0.2$) and submandibular glands (9.4 and 8.0 min, respectively; $p > 0.4$). Peak tracer distribution (C%) was significantly lower in the parotid glands of both the SS (42%) and the IS group (40%) compared to the controls (53%; Fig. 1c; $p < 0.01$). This reduction was symmetric in IS patients, but asymmetric (significant reduction in the left parotid and submandibular glands only) in SS patients (Fig. 1d). Stimulated excretion (E%) was significantly reduced in SS patients (16.3% for parotid and 17.4% for submandibular glands; $p < 0.01$); however, in the IS patients, excretion (32, 2% for parotid and 26, 9% for submandibular glands) was similar from all glands as in the control groups (35, 2% for parotid and 27, 8% for submandibular glands; Fig. 1e and f).

Correlations between SGS data and clinical findings

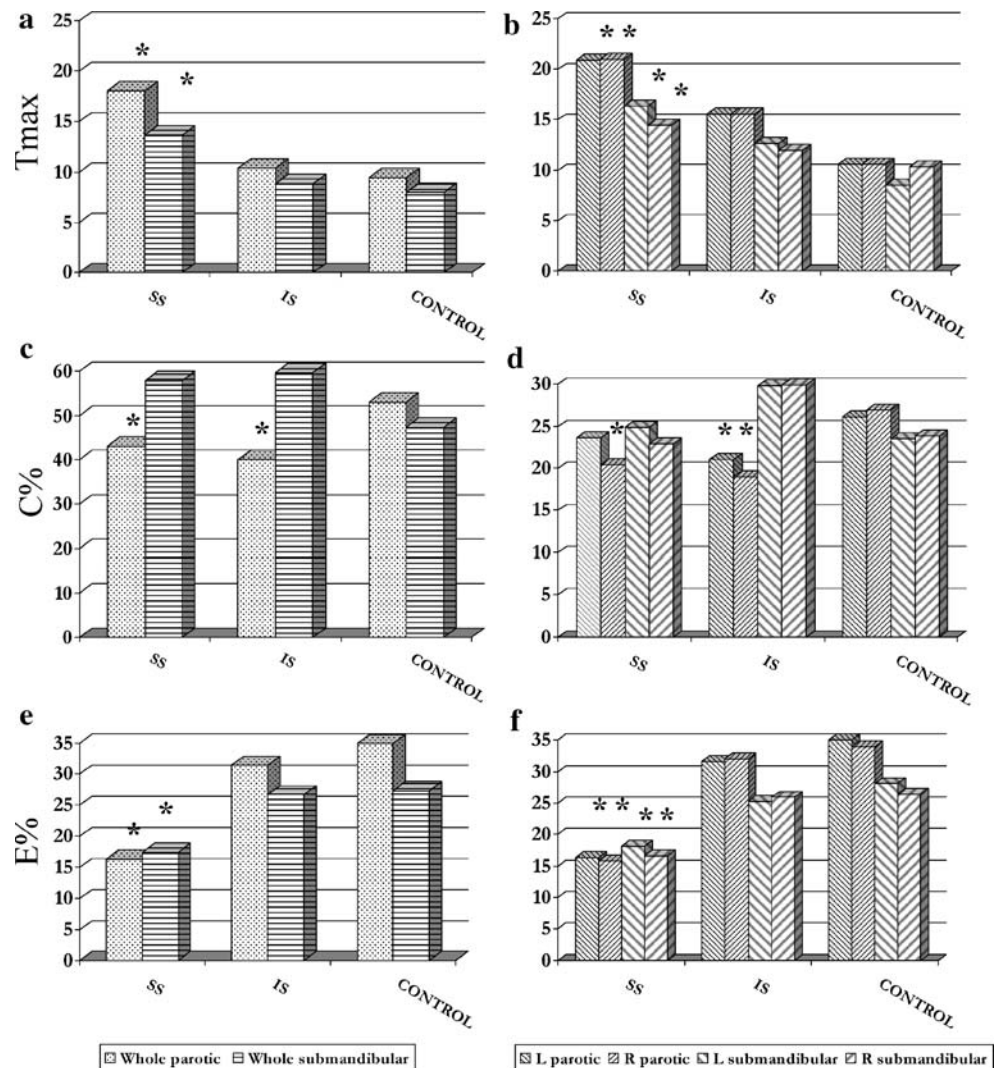
To determine if SGS findings could be related to specific clinical factors, correlation coefficients were calculated between age, focus score, sialometry, ESR, serum levels of creatinin and immunoglobulins and the results of Tmax, C% and E%. No significant correlations were seen within the separate SS or IS groups or within the whole cohort of sicca patients (all *p* values greater than 0.05, data not shown).

Discussion

In this study, a reversed parotid/submandibular uptake ratio, a prolonged Tmax tracer uptake and a reduced tracer secretion especially from the parotid glands distinguished SS patients from controls. Although semi-quantitative SGS data have been studied before, this is one of the few studies using absolute measures for isotope uptake and excretion in a controlled fashion. The degree of reduction in speed and peak uptake and secretion in this study agree to a large extent with the data presented in other studies of paired time-activity curves for submandibular and parotid glands in primary SS patients [8, 13, 14]. Some authors found a reduced uptake in the submandibular glands of SS patients; whereas in the present study, parotid uptake was more affected in SS patients. This discrepancy may be because of methodological aspects as the present SS cohort had shorter duration of sicca symptoms (4 years) compared to long standing diseases (mean 11 years) in the other study. Differing sensitivities of the parotid and submandibular glands to insult over time as reported before may also be one of the explanations [15–17]. Our data indicate a near doubling of the Tmax and a reduction by half of the excretion capacity in SS patients compared to the controls. This illustrates how cut-off levels for absolute values in SGS could become a valuable contribution in SS classification/diagnosis but will require larger prospective studies that standardise tracer administration and salivary stimulation [13].

Delayed tracer uptake and secretion in SS patients is thought to be due to a progressive reduction in exocrine function of the major salivary glands resulting from morphological changes induced by chronic immune-medi-

Fig. 1 Quantitative salivary gland scintigraphy findings in patients with Sjögren's syndrome (SS), isolated sicca (IS) and the control group. Tmax tracer uptake in minutes for the parotid and submandibular glands combined (a) and for each separate gland (b). Peak tracer distribution (C%; percentage of total tracer dose) for the parotid and submandibular glands combined (c) and for each separate gland (d). Stimulated excretion of tracer (E%; given as the percent in reduction of maximum concentration) for the parotid and submandibular glands combined (e) and for each separate gland (f). See "Materials and methods" for more details. Asterisks indicate values that are significantly different from the controls



ated inflammation [1, 3, 18, 19]. This line of thought is, however, mainly based on indirect evidence from minor labial gland biopsy findings in SS. However, normal acinar cells are commonly observed in biopsy materials indicating that dryness cannot be solely explained by glandular destruction. Auto antibodies against muscarinic M3 receptors that inhibit parasympathetic neurotransmission may, however, also contribute to impaired glandular function through the disruption of autonomic function without mediating inflammation [6, 7, 20]. Although we lacked the necessary expertise to measure muscarinic receptor auto antibodies, studying the influence of this new class of auto antibodies on salivation may also profit from quantitative SGS testing.

We did not find a significant correlation between SGS results and anti-SSA auto antibodies, which are found in salivary infiltrates [1, 18]. Although this lack of correlation may be well be due to the low numbers in this study, it could again indicate that factors other than inflammation may also be important in reducing salivation in SS. The exact sequence and contribution of inflammation and dysfunction

to the development of sicca, symptoms are presently unknown; however, quantitative testing of salivary gland function may provide important information not otherwise obtainable. Although unstimulated sialometry is easily available, it remains an indirect measure of salivary gland function as it depends on the integrity of the ductal system. Sialometric findings have a low specificity for SS as various external factors contribute to large variations in flow in healthy cohorts and also within the same individual [2, 21]. A non-invasive test that could replace labial gland biopsy at the salivary test of choice would be a large step forward in classifying/diagnosing pSS, as it would allow both standardisation and inclusion of a much larger proportion of pSS patients in scientific studies. On the condition that quantitative SGS is developed in a standardized fashion, it could ultimately fulfil that goal [13].

There are several limitations to the interpretation of these results. We did not use the latest criteria from the EU-US consensus, as these criteria were not available during the planning stage of this study. Two patients classified as SS

with the use of the preliminary European criteria would not have retained their SS diagnosis with the use of these new criteria set because of the absence of anti-SSA antibodies. In addition, we did not study absolute values for total tracer uptake in our patients. Absolute uptake (i.e. the number of radioactive counts) is a function of salivary gland mass and thus related to body mass. However, these data were not systematically collected in our cohort. Total tracer uptake may be especially interesting in the follow-up of patients with SS as it may reflect the development of gland atrophy. Furthermore, the low number of patients with definite pSS that we were able to recruit and their age difference with controls may have biased our findings. Finally, our control group consisted of patients who were referred for scintigraphy evaluation of suspected nodular thyroid disease. Although all were euthyroid, there have been reports on reduced salivary function in patients with autoimmune thyroiditis [22, 23], and we cannot exclude that subclinical thyroid disease may have influenced our control data. The clinical relevance of distinguishing between IS and pSS lies in the associated risk for extraglandular complications, which occur frequently in pSS but not in IS [1, 2]. This has clear implications for the follow-up and potentially also for the treatment of these patients as confirmed by the lack of progression to pSS in this cohort.

In summary, findings from this controlled pilot study support the use of time activity curves during quantitative SGS as a safe, minimally invasive and objective method to distinguish patients with SS during the evaluation of sicca symptoms. To confirm these results, a cross-sectional study with direct comparison of the diagnostic value of SGS with labial biopsy findings will be needed together with a longitudinal study that investigates the disease course in such cohorts.

Acknowledgement The technical support and advice by Peter van Lom and Professor Johan Sundsfjord at the Department of Nuclear Medicine, University Hospital of North Norway is greatly appreciated.

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