

The prevalence of Sjögren's syndrome and sicca symptoms in patients with systemic sclerosis and alpha-smooth muscle actin expression in biopsy specimens from minor salivary glands

Abstract

Background/Aim: This study aimed to investigate the prevalence of sicca symptoms and secondary Sjögren's syndrome (SjS) in patients with systemic sclerosis (SSc). Also this study aimed to evaluate the expression of α -smooth muscle actin (α -SMA) in minor salivary gland (MSG) specimens, a possible marker of fibrosis responsible for myofibroblastic transformation.

Materials and methods: Patients with SSc who were followed in Rheumatology outpatient clinic at a university hospital evaluated. The questionnaire of sicca symptoms and classification of SjS were evaluated according to the American-European Consensus Group (AECG) criteria. Histopathologic evaluations were done in MSG specimens investigating the presence of focal lymphocytic sialadenitis and glandular fibrosis, also assessing the expression of α -SMA.

Results: This cross-sectional study included 102 patients with SSc [91 female (89%), mean age 52.5 ± 12 years]. In this cohort 76 (75%) patients had sicca symptoms and 36 (35.3%) patients fulfilled the AECG criteria for SjS; all with limited form. Having SjS found to be associated with older age and the presence of positive anti SS-A antibodies. On histopathologic examinations, glandular fibrosis

was observed in 67 (80%) and lymphocytic sialadenitis was detected in 38 (45%) patients; but only 7 samples were positive for α -SMA.

Conclusion: This study suggested sicca symptoms were found to be very common among patients with SSc. Also secondary SjS was detected in nearly one-third of patients with SSc; especially in limited subtype. Anti SS-A positivity and older age were detected as predictors for SjS. Histopathologic evaluations showed significant glandular fibrosis but rare α -SMA staining in patients with SSc.

Key words: Fibrosis, scleroderma, sicca, Sjögren's syndrome, alpha-SMA

1. Introduction

Scleroderma (SSc) is a heterogeneous systemic disorder characterized by massive accumulation of collagen and matrix proteins in connective tissue [1]. SSc may also co-exist with Sjögren's syndrome (SjS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) or polymyositis (PM) at variable rates and this condition is referred to as overlap syndrome.

SjS is a chronic autoimmune disorder that predominantly affects exocrine glands. Essential histological characteristic of SjS is focal lymphocytic infiltration of salivary and lacrimal glands. The rate of secondary SjS has been found, as 17%-19% in previous publications while extreme rates such as 1%-90% have also been

reported among patients with SSc [2]. Sicca symptoms such as xerostomia or xerophthalmia are also common in patients with SSc [3]. The condition that leads to hypofunction of exocrine tissues such as salivary glands in SSc has been commonly linked to glandular fibrosis and in certain studies; it has been linked to coexistence of SjS and SSc [4]. In a previous study, subjective xerostomia was reported in 68% of the patients with SSc and histological analysis of minor salivary gland (MSG) biopsies revealed 'fibrosis' in 55% of these patients [4].

The main characteristic of the SSc pathogenesis is pathological fibrosis: excessive deposition of extracellular matrix (ECM) induced by activated fibroblasts [5]. During the tissue repair process, fibroblasts and other progenitor cells differentiate into myofibroblasts in response to extracellular signals that is followed by the induction of collagen synthesis, the synthesis of other ECM proteins, connective tissue adhesion and contraction, the release of growth factors. Under physiological conditions, the repair program is a self-limited, strictly controlled process. However, pathological fibrosis is characterized by continuous and increased fibroblast activation, which results in excessive ECM deposition and remodeling. Cells affected by SSc during the earlier stages include endothelial cells, pericytes, vascular smooth muscle cells and immune cells in the perivascular spaces. Among these cells, only endothelial cells are mesenchymal cells subject to apoptosis, however pericytes and smooth muscle cells rapidly proliferate [6]. Pericytes have the potential to differentiate into vascular smooth muscle cells, fibroblasts and myofibroblasts [specialized contractile cells able to secrete α -smooth muscle actin (α -SMA) and fibronectin ED-A variant] and may affect endothelial cell proliferation. Experimental

studies indicate that myofibroblasts may play a central role in the development of fibrosis by expressing α -SMA in patients with SSc [7].

Therefore, this study purposed to determine the prevalence of sicca symptoms in patients with SSc, also to determine the patients meeting the classification criteria of secondary Sjögren's syndrome, based on the American-European Consensus Group (AECG) Criteria for SjS [8]. Then, this study aimed to assess ' α -SMA' expression in MSG specimens in this patient group with SSc and SjS, as an indicator of myofibroblastic transformation, in order to investigate the contribution of this process to the fibrosis.

2. Materials and Methods

This cross-sectional study included 102 patients with SSc who were diagnosed and followed-up in the Rheumatology outpatient clinic at Dokuz Eylül University Medical Faculty (İzmir, Turkey). All the patients with SSc met the 1980 American College of Rheumatology criteria for SSc or the classification of early SSc [9, 10].

The patients with SSc were consecutively selected. Patients were excluded from the study if they had prior therapy of head and neck irradiation, hepatitis C virus (HCV) infection, adult immune-deficiency syndrome (AIDS), lymphoma, sarcoidosis, immunoglobulin-G4 related disorders, graft-versus-host disease (GVHD) and a history of anticholinergic medication use within 4 half-lives of the agent prior to enrollment.

Basic demographics, clinical and laboratory data obtained from each patient were recorded on a standard form. The physical findings, previous medications, the period of disease and symptoms were all recorded. Complete blood count (CBC), the serum renal and liver function tests, serum albumin, globulin, protein, creatine kinase (CK) levels, serum lactate dehydrogenase (LDH), urinalysis, viral serology assessments for HIV and HCV, serum C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) assessments were all recorded.

Immunological assessments with antinuclear antibody (ANA), Rheumatoid factor (RF), anti extractable nuclear antigen (anti-ENA) screen (including anti SS-A, anti SS-B, anti U1-RNP, anti Jo-1, anti-Sm, anti-Scl 70) were all evaluated. ANA were assessed with immune fluorescein assay; measurement the titer of $> 1/160$ was accepted positive in the study. Anti ENA screen were assessed with immunblotting. RF was evaluated with immunoturbidimetric determination method. The level of serum RF > 14 mg/dl was accepted as positive.

The diagnosis of ILD in patients with SSc is based on the presence of the characteristic chest high-resolution computed tomography (HRCT) features of ILD and exclusion of other etiologies of pulmonary parenchymal disease. SSc-associated PAH is defined as a mean pulmonary artery pressure (PAP) greater than 20 mmHg in right heart catheterization, a wedge pressure less than or equal to 15 mmHg, and a peripheral pulmonary vascular resistance (PVR) ≥ 3 Wood units in patients with SSc.

The presence of subjective dry eye (xerophthalmia) and dry mouth (xerostomia) symptoms was investigated in all patients as defined in the AECG Classification Criteria for SjS [8]. For patients whose responses were affirmative, the intensity of dry eye and dry mouth symptoms were measured using a 0 to 10 Visual Analogue Scale (VAS).

All subjects underwent the Schirmer 1 Test, as an objective diagnostic test for dry eye. The Schirmer 1 test results were considered positive if the wetness of a filter paper measured ≤ 5 mm [11]. Subjects also underwent the unstimulated whole salivary flow test (UWSF), one of the objective indicators of dry mouth. UWSF was performed after 2 hours of fasting. A falcon tube was used as a graduated container to accumulate the saliva and a saliva flow rate of ≤ 1.5 ml/15 minutes was interpreted as abnormal [12].

A MSG biopsy was performed based on either (Figure 1):

- The presence of subjective sicca symptoms according to the AECG criteria set,
- An abnormal Schirmer 1 test,
- A positive UWSF test at the baseline.

Since the patients in our study had SSc, Sjögren's syndrome was classified as defined by the AECG criteria for secondary SjS [8].

2.1 Histopathological analysis

The same experienced pathologist assessed MSG biopsy materials. All slides were assessed for surface area, intensity of lymphocytic infiltration, number of foci, the presence of any giant focus, lobular and acinar atrophy, non-specific sialadenitis and location and intensity of fibrosis. Histological demonstration of a focal lymphocytic sialadenitis, a focus score (inflammatory infiltrate characterized by the presence of 50 or more lymphocyte) ≥ 1 (grade 3 or 4 in a 4 mm² area of the salivary gland tissue based on Chisholm and Mason scale) was defined as diagnostic for SjS [13]. Fibrosis was graded as mild, moderate and severe.

2.2 Immunohistochemical Analysis

In biopsy specimens from MSG, α -SMA expression was assessed by immunohistochemistry. For this purpose, sections prepared on poly-L-lysine coated slides were stained by actin (Actin Smooth Muscle (ASM-1)-alpha Mouse/Afpur IB; IHP; IF Hu, 25 uq, Quartett). An experienced pathologist assessed the sections by using a light microscope. Positive staining of myoepithelial cells and smooth muscles of vessel walls provided an internal control for each case. Expression of α -SMA was considered 'positive' when findings in stromal region were positive in non-epithelial areas of tubule-alveolar region.

2.3 Ethics

The Dokuz Eylül University Hospital Ethics board approved the study with the number of B.30.2.DEU.0.01.00.00/13529. Every patient who agreed to participate in the study provided a written informed consent.

2.4 Statistical analysis

The continuous variables were presented as means and standard deviations and categorical variables were presented as percentages. A non-parametric Spearman's test was used to analyze inter-variable correlations and the multivariate logistic regression test was used to detect variables to be used to predict the development of SjS. A SPSS V.11.0 (SPSS Chicago, Illinois, USA) database was used to conduct all study analyses. The statistical significance level was defined as p values less than 0.05.

3. Results

There were 102 patients with SSc [91 (89%) females; mean age 52.5 ± 12 years] who followed-up in the rheumatology outpatient clinic. The mean age at the onset of the disease was 42.3 ± 14.8 years and the mean disease duration was 10.9 ± 9.8 years. The majority of patients had limited SSc subtype (LSSc) [85 patients (83%)]. Other subgroups were diffuse SSc (dSSc) [12 patients (12%)] and sine SSc (sSSc) [5 patients (5%)].

In our study group the mean ESR was 31.4 ± 18 mm/h and the mean serum CRP level was 9.1 ± 16.5 mg/L. Ninety-five patients (93%) tested positive for ANA. The most common ANA staining pattern was the homogenous pattern with a frequency of 48%. Other ANA types detected in the specimens and their distributions were as follows: centromeric (34%), nucleolar (8%), granular (5%). Anti-ENA was positive

in 49% of the patients. Anti Scl-70 was positive in 38% of the patients. Anti SS-A antibody was positive in 13 patients antibodies, alone or with other autoantibodies. Anti SS-B was positive in only one patient who also tested positive for anti SS-A antibodies (Table 1). Also 20 patients (20%) had pulmonary arterial hypertension (PAH) and 35 patients (34%) had interstitial lung disease (ILD) (Table 2).

A structured questionnaire administered to the patients revealed that 76 out of 102 (75%) patients had subjective sicca symptoms [xerophthalmia in 49 (48%) and xerostomia in 65 (64%)]. The Schirmer 1 test was positive in 65 patients (64%) and the UWSF was positive in 36 patients (35%). A MSG biopsy was indicated in 90 patients according to the study protocol. Six out of these 90 patients refused to undergo a biopsy and a total of 84 patients underwent a biopsy (Figure 1).

In histopathologic analysis, fibrotic lesions were observed in the specimens from 67 patients (80%) while the focus score was ≥ 1 in 38 (45%) patients (Figure 2). The rates of patients with mild, moderate and severe fibrosis were 67%, 24% and 9%, respectively.

Consequently, 36 (35.3%) patients were classified as having secondary SjS according to the AECG classification criteria. Twelve patients with secondary SjS had a focus score of <1 in MSG biopsies.

The SSc patients of whom had secondary SjS, 34 had LSSc and 2 had sine scleroderma. In patients with SSc and SjS, none of the patients had dSSc. In patients

with diffuse SSc, histological analysis of biopsy specimens from six patients revealed glandular fibrosis at variable rates while one of these specimens exhibited severe fibrosis. Glandular fibrosis was seen in 6 patients with dSSc (50%) and 61 patients with LSSc (71.8%) (p: 0.18).

Immunohistochemical analyses revealed that only 7 biopsy specimens exhibited a weak staining with α -SMA. All of these 7 patients had LSSc subtype of SSc (Table 3). Among specimens positive for α -SMA staining, the focus score was ≥ 1 in salivary gland specimens from 3 patients and all of them exhibited glandular fibrosis ranging from mild to severe in intensity.

The presence of secondary SjS found to be moderately correlated with age, age at the onset of symptoms and having positive anti SS-A [correlation coefficient (r) were r: 0.407 (p < 0.0001); r: 0.343 (p < 0.0001) and r: 0.270 (p: 0.006), respectively] and weakly correlated with the presence of positive RF [r: 0.216 (p: 0.035)].

In our study group, no associations were found between the presence of secondary SjS and other clinical and laboratory parameters including positive ANA, anti-Scl70 and anti-centromere antibodies, having PAH, high levels of ESR and serum CRP.

In patients with SSc, the factors of age, age at the onset of symptoms, positive SS-A antibodies, positive RF, having PAH and ILD were evaluated as possible risk factors for secondary SjS. In univariate logistic regression analyse, age, age at the onset of symptoms, positive SS-A were found related with secondary SjS. In multivariate

logistic regression analyse, only two variables were statistically significant independent predictor factors for the development of secondary SjS: age and positive SS-A (Table 4).

In this study a significant association was found between older age and glandular fibrosis in minor salivary gland specimens (p: 0.034), however no association was found between the presence of fibrosis and other clinical and laboratory parameters.

4. Discussion

This study showed, the prevalence of sicca symptoms was found to be very common among patients with SSc (75%). Also secondary SjS was detected in nearly one-third of the patients with SSc (35.3%), especially in the limited subtype of SSc. The main predictors of having secondary SjS were detected as anti SS-A positivity and older age in patients with SSc. The patients with SSc frequently encountered with sicca symptoms so these findings were important in understanding the pathogenesis of this condition.

The association of SSc and SjS has been reported in a range varying from 1 to 90% in previous studies many of which were very old. This wide range of prevalence might depend on heterogeneity of the definitions for the SjS. More than ten classification criteria sets have composed for SjS since today. One of the highest prevalence of secondary SjS has been reported by Alarcon-Segovia et al., as 90% in

patients with SSc [2]. They analyzed all the objective and subjective tests for SjS, Schirmer 1, Rose Bengal tests, parotid sialography and scintigraphy, MSG biopsies and then accepted patient as secondary SjS if either of them positive. The prevalence of SSc-associated SjS was reported as 69% by Spanish investigators [14]. In this study Coll et al., defined secondary SjS as, two out of three criteria were prerequisite to define SjS in patients with SS included xerostomia (based on MSG biopsy or scintigraphy), xerophthalmia (based on subjective irritation and burning sensation, the Schirmer 1 and the Rose Bengal test) and having autoimmune disorders [14]. Cipoletti et al, classified SjS based on the findings from MSG biopsies, independently from clinical manifestations and the prevalence of SjS was found to be 17%, which was lower than those reported from other studies [15]. In another study from Greece, the prevalence of SjS was found to be 20.5% in 44 patients with SSc [16]. A positive MSG biopsy and the presence of xerostomia (subjective symptoms or reduced parotid salivary flow rate) or xerophthalmia (a positive Rose Bengal test) were the prerequisites for a diagnosis of SjS in this study. The Copenhagen criteria were used to diagnose SjS in a study conducted in patients with SSc and only one patient was diagnosed with secondary SjS [17]. There was one study using the AECG criteria to investigate the prevalence of SSc-associated SjS before. In this prospective study of Avouac et al., the prevalence of SjS among patients with SSc was found to be 14% [4].

In our study the AECG criteria [8] was preferred to use, which covers both subjective and objective items; and at least one serological/histopathological item needed to be classify a patient as having primary SjS. And the AECG criterion was preferred

because it was one the most commonly used validated criteria set in the studies of SjS. In our study the prevalence was detected 35.3% for secondary SjS in patients with SSc. This was a higher prevalence than the Avouac et al., who used also the AECG criteria; however this study did not comply with the 5th item (UWSF) of this criterion set, as stated by the authors and this might have contributed to the low SjS prevalence reported in this study.

The distribution of the different subtypes of the SSc might also play a role in the variability of the SjS prevalence rates reported from the studies in the literature. All patients with SjS in our study had limited cutaneous form of scleroderma and these data were consistent with previous data indicating that LSSc might be a risk factor for the development of SjS [3, 4]. In our study although a statistically significant association was found between SjS and the limited cutaneous subtype of scleroderma in correlation analysis (p: 0.024), this result should be interpreted with caution since none of the patients with dSSc had SjS. This observation might be the result of underrepresentation of dSSc (only 12% of the subjects) in our cohort. The low ratio of dSSc patients in our study also contributed to our inability to demonstrate any associations between the development of SjS and different autoantibodies such as anti-centromere autoantibodies or anti-scl70 antibodies.

The reasons for the propensity to SjS in patients with LSSc have remained unknown, as similar with primary biliary cirrhosis (PBC) that seen more in LSSc. Studies have pointed out the potential role of Th2 polarization due to the altered B-cell homeostasis including increased naive B-cell counts and reduced memory B-cells counts as well as increased CD4⁺ and CD30⁺ T-cell counts and significantly

elevated levels of serum soluble CD30 in skin biopsy specimens [18, 19]. These alterations may be observed more commonly in patients with LSSc, in particular when compared to dSSc patients with proven clonal expansion of T-cells [4]. This might also explain the propensity to other autoimmune diseases in patients with LSSc.

Although the high prevalence of sicca syndrome in systemic sclerosis is a well-known fact, underlying etiological factors are not clear. Several studies have reported that fibrotic changes in salivary glands might be responsible for these signs and symptoms [2, 4]. In our study, 62 patients had xerostomia and underwent a MSG biopsy. Having SSC and SjS simultaneously could interpret these symptoms in half of the patients (31 patients). The xerostomia of the remaining patients might be explained by having fibrosis in MSG tissues; the rates of patients with mild, moderate and severe fibrosis in MSG were 67%, 24% and 9%, respectively. These findings were similar with the data reported that xerostomia in patients with SSc might be related to glandular fibrosis or secondary to SjS [20]. In our study older age ($p: 0.034$) was detected associated with MSG fibrosis but no associations were found between MSG fibrosis and clinical signs and symptoms and laboratory assessments. The focus score was ≥ 1 in 24 patients among patients having SjS and 21 out of these 24 patients also had glandular fibrosis. Among 62 patients with SSc who exhibited xerostomia, a total of 51 had fibrotic changes. So the fact that fibrosis did not correlate with clinical findings might be due to its widespread prevalence in patients with SSc. Our data showed that it is clear that the contribution of fibrosis cannot be denied in the presence of sicca symptoms in patients with SSc and also SSC/SjS.

In our cohort 25% of the patients with SSc and secondary SjS had detected positive for anti SS-A antibody. The prevalence of anti SS-A in patients with SSc reported as similar in some previous studies [16], as well as higher rates of 53% to 60% also reported by some researchers [21]. In our study there was a correlation between the presence of anti-SS-A antibodies and co-existence of SjS in patients with SSc. Also the multivariate regression analysis affirmed that the presence of anti- SS-A was the sole determinant for secondary SjS, along with the age. This finding suggested that anti SS-A antibody strongly predicts the development of SjS in patients with SSc.

Likewise the previous studies revealed that the rate of patients who had positive RF was higher among patients with SjS/SSc overlap syndrome [22]. Although the presence of RF was correlated with the development of secondary SjS ($r: 0.216$, $p: 0.035$) in our study, it was not an independent variable based on regression analysis.

Some previous studies showed an association between clinical features of SSc and having secondary SjS. For example the results of three studies [3, 4, 22] demonstrated that the presence of SjS in association with SSc might be protective against SSc-associated pulmonary fibrosis. In these studies, SjS was more prevalent in a group of patients with LSSc subtype. Also the presence of SjS in association with SSc might be protective against SSc-associated pulmonary arterial involvement [22]. In our study, the groups were detected similar in terms of systemic organ involvements. Also some authors suggested that there was a distinct SSc patient subgroup that had anti centromere antibody (ACA) positivity and SjS overlap, with

clinical features as mild SSc phenotype but more frequently seen of lymphoma [23]. Very recent imaging studies also supported this entity by showing similarities in ultrasonography of salivary glands (SGUS) between patients with primary SjS and patients with ACA positive-LSSc [24]. Our findings supported this subtype by showing the high prevalence of SjS in patients with LSSc. With this growing observational data, a distinct clinical syndrome might be defined in near future including the patients with LSSc and SjS overlap.

The main characteristic of the SSc pathogenesis is pathological fibrosis; excessive deposition of ECM induced by activated fibroblasts. The cells that play a major role in fibrogenesis were increased mesenchymal cells, which had ability of differentiation between myofibroblasts, fibroblasts and contractile smooth muscle cells [25]. During the fibrotic process, activated mesenchymal cells in tissue promote ECM. Myofibroblasts that are specialized cells that synthesize collagens; tissue inhibitors of metalloproteinases and other ECM components are both the main source of TGF- β and also transdifferentiate from fibroblasts in response to TGF- β . Myofibroblasts express a cytoskeleton protein, α -SMA [26]. Other than the dermis, the expression of TGF- β , α -SMA and other cytokines was also demonstrated by immunohistochemistry in biopsy specimens from the stomach walls of patients with SSc [27]. There are very limited information about the pathogenesis of fibrotic lesions of pulmonary tissue and others in SSc. Therefore, in this study, we also investigated whether or not myofibroblastic transformation, which was known to contribute to skin lesions of SSc, had a role in the occurrence of sicca symptoms. However a weak immunohistochemical staining with α -SMA was detected in

specimens from 7 patients. Fibrotic changes were detected in all of these specimens and the focus score was ≥ 1 in specimens from 3 patients. However these results suggest that the α -SMA expression and thereby myofibroblasts might not play a role in the occurrence of sicca symptoms or in the development of SjS. Similar as our study, measurement of α -SMA in other tissues such as skeletal muscle was not associated with for fibrosis (for familial muscular dystrophies) [28]. But Sisto et al. suggested opposite findings about α -SMA in a study comparing patients with pSS and healthy people. They suggested disappearing of α -SMA might be one of the disturbance associated with disease pathology [29]. The weak expression of α -SMA might contribute to the loss of myoepithelial cells and mechanical support concluded with the reduction of salivary gland flow and xerostomia.

As a limitation, in our study there was not a group of healthy subjects or primary SjS patients for evaluating the measurement of α -SMA. A further study including a control group for evaluating α -SMA is needed to confirm the interpretation of histopathologic findings.

In conclusion, this study showed the prevalence of sicca symptoms was found to be very common, and secondary SjS was seen in nearly one-third of patients with SSc. These findings suggest that the association between SSc and SjS was not coincidental and unknown common factors might play a role in the pathogenesis. Especially the limited subtype of patients with SSc was found to be associated with SjS. This study showed that it is clear that the contribution of fibrosis cannot be denied in the presence of sicca symptoms in SSc patients with or without secondary

SjS. But further investigation into the α -SMA needed to define its role in the mechanisms of salivary gland dysfunction.

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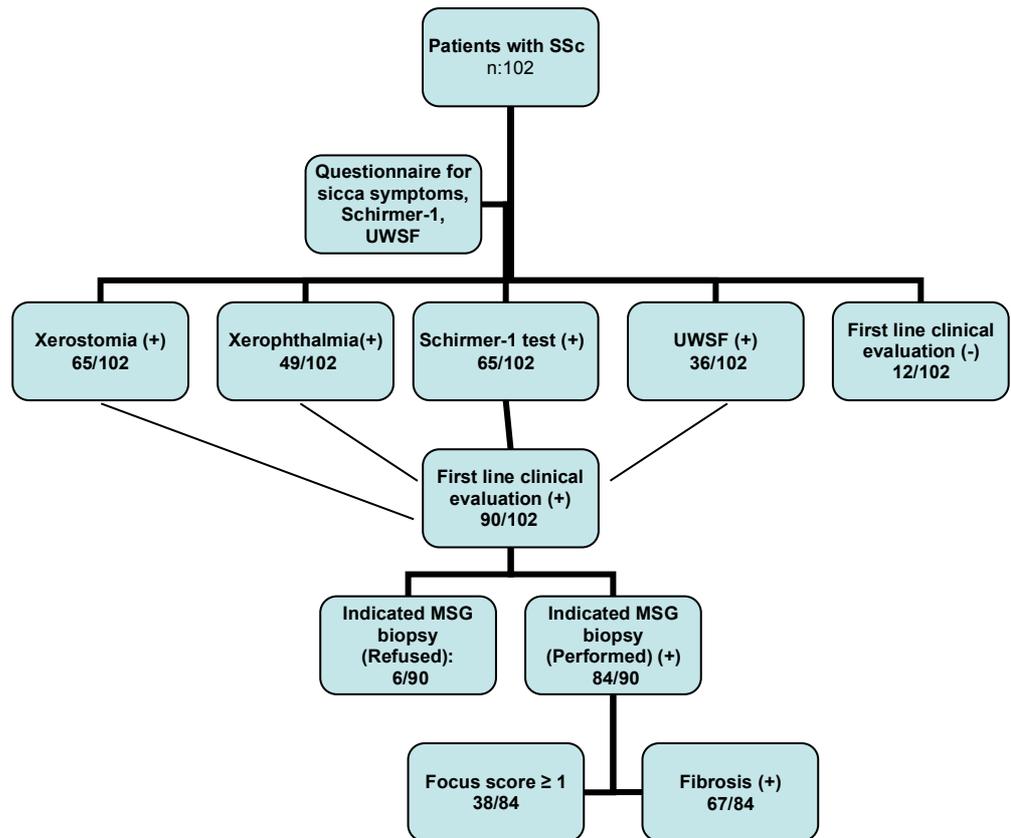


Figure 1. Overview of the methodology used for the study

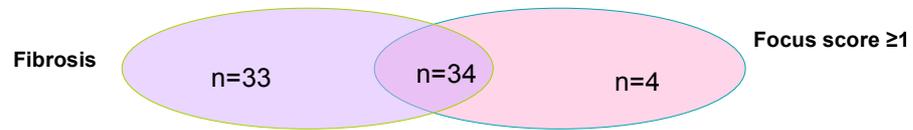


Figure 2. The findings of histopathologic evaluations of MSG biopsy specimens
MSG: minor salivary gland; n: number.

Table 1. The clinical and laboratory data of the patients with SSc

Parameter	
Age (years); mean \pm SD	52.5 \pm 12
Sex, female; n (%)	91 (89%)
SSc subtype	91 (89%)
LSSc; n (%)	85 (83%)
dSSc; n (%)	12 (12%)
sSSc; n (%)	5 (5%)
Disease duration (years); mean \pm SD	10.9 \pm 9.8
ESR (mm/h); mean \pm SD	31.4 \pm 18.0
CRP (mg/L); mean \pm SD	9.1 \pm 16.5
ANA positivity; n (%)	95 (95%)
ANA (centromeric); n (%)	34 (34%)
Anti SS-A positivity; n (%)	13 (13%)
Anti Scl-70 positivity; n (%)	38 (38%)

SSc: Systemic sclerosis; dSSc: diffuse form systemic sclerosis; LSSc: localized form systemic sclerosis; sSSc. Sine scleroderma; ESR: erythrocyte sedimentation rate; CRP: serum C-reactive protein; ANA: anti nuclear antibody. SD: standard deviation

Table 2. The clinical features and the organ involvements of the patients with SSc

Clinical feature	
Modified Rodnan score; mean \pm SD	10.4 \pm 8.4
Interstitial lung disease; n (%)	35 (34%)
Pulmonary arterial hypertension; n (%)	20 (20%)
Digital ulcers n (%)	19 (19%)
Digital gangrenes; n (%)	3 (3%)
VAS score; mean \pm SD	4.7 \pm 2.3
Functional score; mean \pm SD	6.8 \pm 7.8

SD: standard deviation; VAS: visual analogue scale

Table 3. The clinical features of the patients with SSc who had positive α -SMA in MSG biopsies

Patient	Focus score ≥ 1	Fibrosis	Schirmer (+)	UWSF (+)	Lung fibrosis	PAH	ANA Pattern	Anti-ENA screen
1.	+	Moderate	+	+	-	-	Centromeric	Negative
2.	+	Serious	+	+	-	NA*	Centromeric	Negative
3.	+	Mild	+	-	+	-	Nucleolar	Negative
4.	-	Serious	+	-	-	+	Nucleolar	Negative
5.	-	Moderate	-	-	+	-	Homogenous	Anti Scl-70
6.	-	Mild	+	+	-	+	Nucleolar	Negative
7.	-	Moderate	+	-	+	-	Nucleolar	Negative

SSc: scleroderma, SjS: Sjögren's syndrome; α -SMA: Alfa-Smooth Muscle Actin;

UWSF: unstimulated whole salivary flow; PAH: pulmonary arterial hypertension;

ANA: antinuclear antibody, NA: not available

Table 4: Results of multivariate logistic regression analyse for risk factors affecting having SjS in patients with SSc

Risk factor	P value	OR (95% CI)
Age	0.038*	0.109 (1.004-1.033)
Age at the onset of symptoms	0.062	1.020 (0.999-1.043)
Positive SS-A	0.015*	3.211 (1.310-5.980)

OR: odds ratio, CI: confidence interval