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ORIGINAL ARTICLE

Systemic sclerosis: demographic, clinical and serological features in 100 Iranian patients

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Abstract To evaluate demographic, clinical and laboratory features associated with scleroderma-specific auto-antibodies. Sera of 100 patients with systemic sclerosis (SSc) were analyzed by an indirect immunofluorescence technique with HEp-2 cells as a substrate. Specific ANA such as anti-centromere antibodies (ACA), anti-topoisomerase (TOPO), anti-RNA polymerase III (Pol 3), anti-U3-RNP (U3-RNP), anti-Th/To (Th/To) and anti-PM/Scl (PM/Scl) were detected by line immunoassay and anti-U1-RNP (U1-RNP) by ELISA. Frequency of clinical features associated with a specific antibody group was reported cumulatively over the follow-up period. Frequency of specific clinical features was compared across the two disease subtype including limited cutaneous (lcSSc) or diffuse cutaneous (dcSSc) as well as the auto-antibody groups. Ninety-four percent of patients were ANA positive with significant higher skin score, Raynauds and digital ulcer/gangrene. Anti-TOPO was detected in 71 % of all patients, in 90.5 % of dcSSC and in 65.8 % of lcSSc. Anti-TOPO was significantly associated with dcSSc, higher skin score, digital ulcer/gangrene, pulmonary fibrosis, DLCO <70 %. U1-RNP antibody was associated with lower fibrosis in lung. ACA was positive in 7 % of patients and exclusively in those with lcSSc. We did not find association between gender and presence of auto-antibodies. Anti-TOPO antibody had a high prevalence in contrast to low prevalence of ACA antibody. There were no differences in clinical subtypes of the disease in patients with positive anti-TOPO and positive ACA. Differences in prevalence of auto-antibodies are suggestive of further genetic study.

Keywords Systemic sclerosis · Auto-antibodies · Disease subsets

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Introduction

As in other autoimmune diseases, in systemic sclerosis (SSc), the presences of auto-antibodies have been shown in more than 95 % of patients [1]. There are at least 7 disease-specific auto-antibodies that are not known whether have a direct role in pathogenesis of SSc or their presence is an epiphenomenon [2].

The correlation between some of the specific antibodies with disease subsets and specific features of SSc has been demonstrated. Typically, the anti-scl-70 (anti-topoisomerase I/TOPO) and anti-centromere antibodies (ACA) were closely related to clinical manifestations of two major subsets of the disease namely limited (lcSSc) and diffuse cutaneous scleroderma (dcSSc) [3].

Moreover, association of interstitial lung disease (ILD) with anti-TOPO; renal crisis with anti-RNA polymerase III (Pol3); digital necrosis and primary pulmonary hypertension with ACA; anti-U3 RNP/fibrillarin, anti-Th/To [4], anti-U1 RNP and anti-PM/Scl with overlap syndrome have been shown [5].

Previous studies demonstrated variations in distribution of auto-antibodies and clinical features of SSc. For example, in different parts of the world, prevalence of ACA reported from 11 to 42 %, whereas the frequency of anti-TOPO from 9.4 to 42 % [4].

Genetic, ethnic and environmental factors might explain such variations.

The purpose of the current study was to evaluate demographic and clinical features associated with seven scleroderma-specific antibodies for the first time in Iranian patients and in Middle Eastern population. We also have studied correlation of scleroderma-specific antibodies with clinical features, the two disease subsets (lcSSc and dcSSc) as well as early and late stages of the disease [3].

Patients and methods

Clinical assessment

Hundred patients with initial visit to Firoozgar Hospital, a university affiliated hospital from February 1988 to September 2010, were included. After obtaining an informed written consent, Sera were taken and stored. All patients underwent a standard baseline evaluation and mostly had regular follow-up.

In current study, the patients' data were obtained from Clinical and Research Rheumatology Information System (CRIS) software which was designed locally and has been used since 2008. A longitudinal electronic data bank, CRIS is an electronic data recording software that is used to register and follow up patients' demographic, clinical and paraclinical data at the baseline and throughout the study. The software also has a capacity to save scanned radiographical images that allows prospective follow-ups.

Disease onset was defined as the date of first symptom attributable to scleroderma, for example, Raynaud's phenomenon, swollen fingers and renal crisis. Organ involvement and clinical manifestation of disease is defined as shown in Table 1.

We first evaluated basic demographic and clinical features in different disease subsets; lcSSc and dcSS then looked into correlation of demographic and clinical features with different auto-antibodies. Disease subsets classification was based on extension of skin involvement as defined by LeRoy et al. [6].

Additionally, to study the correlation of antibodies with different disease stages, patients were divided into two groups with early and late stages of disease. The disease duration was defined based on the time of first attributable symptom to systemic sclerosis. For dcSSc subset, duration of <3 years and >6 years was classified as early and late stages, for lcSSc subset, duration of <5 years and >10 years classified as early and late stages of disease retrospectively [8].

Serologic studies

Indirect immunofluorescence antibody (IIF) was carried out using Bio-chip mosaics with Hep-2 cells and primate liver as substrate (Euroimmune kit, Germany) [9].

All sera were tested with serial dilutions ranging from 1/40 to 1/600. Titers above 1:100 considered positive. Two independent observers reported the pattern of positive samples by using Olympus fluorescent microscope. In case of disagreement between the two observers, an expert immunologist become involved and commented.

ANA IIF test results are usually reported in systemic sclerosis based on speckled, centromere or anti-nucleolar pattern.

We used line immunoassay [Euroline systemic sclerosis profile (IgG), Euroimmune, Lubeck, Germany], a test kit with coated strips and parallel lines of highly purified antigens of anti-centromere (CENP-A, B), RNA Polymerase III

Table 1 Definition of organs involvement in systemic sclerosis

Clinical features	Definition
Peripheral vascular	Raynaud's phenomenon observed by a physician, digital pitting ulcer, telangiectasia and ulceration or gangrene
Skin	Puffy hands and skin score based on modified Rodnan Skin Score. Disease subsets classification was based on extension of skin involvement as defined by LeRoy et al. [6]
Joint	Polyarthralgia or arthritis in more than one joint, carpal tunnel syndrome or palpable tendon friction rub
Skeletal muscle	Myositis was defined as proximal muscle weakness on physical examination and any of the following: Muscle biopsy showing myositis, electromyogram with a myopathic pattern or elevated serum enzymes reflecting a muscle disease
Gastrointestinal	History of esophageal reflux, diarrhea or constipation
Pulmonary	Interstitial lung disease was defined as bilateral basilar fibrosis on chest radiography or high resolution computerized tomography (HRCT) scans and/or restrictive pattern on pulmonary function test, that is, forced vital capacity (FVC) of less than 70 % of predicted value. Isolated pulmonary hypertension was defined as pulmonary artery systolic pressure >45 mmHg without interstitial fibrosis. This threshold value has a 97 % association with PAH at catheterization [7]
Cardiac	Pericarditis and symptomatic left ventricular heart failure and/or arrhythmia requiring treatment
Renal	Scleroderma malignant hypertension and/or rapidly progressive renal insufficiency and/or microangiopathic hemolytic anemia

(Pol3), (RP11, 155), Scl70 (TOPO), fibrillarin/U3 ribonucleoprotein (anti-U3 RNP), PM/Scl (75,100) and Th/To).

At first, step-diluted serum samples were incubated with immunoblot strips in positive and the specific IgG (also IgA, IgM) bond to the corresponding antigen site. The bound antibodies were detected by the second incubation and carried out by using an enzyme-labeled anti-human IgG (enzyme conjugate) which catalyzed a color reaction. The reaction intensities were automatically evaluated by a computer program named Euroline Scan. A cutoff value of 10 intensity units in Euroline scan program considered as a positive result. Line immunoassay can be used as a valuable tool and a substitute for Enzyme-Linked Immunoabsorbant Assay (ELISA) [10].

We used Orgentec kit for detecting anti-U1-RNP-70 antibody by applying ELISA technique according to the manufacturer's instructions.

Statistical analysis

Chi square analysis was performed to compare categorical demographic data and auto-antibodies prevalence in different disease subsets. If dependent variables had a normal distribution, continuous variables were analyzed by *t* test. Where *t* test was not applicable, Mann–Whitney *U* test was used. We used univariate ANOVA to compare continuous variables in more than two groups. Multiple logistic regression analyses also were performed on clinical features and auto-antibodies prevalence (independent variables) and on disease subsets or stages (dependent variables). Correlation between ANA pattern and specific antibodies was determined by using Crammer V test. All statistical tests were performed applying SPSS V.10 statistical package. Correlation between ANA pattern and specific antibodies was determined by using Crammer V test. All statistical tests were performed applying SPSS V.10 statistical package.

Results

Baseline demographic and clinical presentation in different disease subsets

Seventy-nine patients were classified as having lcSSc subset of disease and 21 as dcSSc (lcSSc/dcSSc ratio was 3.4/1). Female/male ratio was 6.7–1 with 87 of patients being female. There was no statistical significance in gender ratio when we compared the two disease subsets ($p = 0.54$). Patients' mean age did not differ between the two subsets of disease as shown in Table 2. Skin score's mean rank (modified Rodnan Skin Score, mRSS) was higher in dcSSc (82.7) compare to lcSSc (41.9), ($p = 0.001$).

We did not find any statistical significance when compared clinical and paraclinical features of the two disease subsets (Table 2); however, we could find numerical differences. Some examples include: Raynaud's phenomenon observed by physician was reported in 68.4 % of lcSSc group compared to 52.4 % in diffuse subset. Carpal tunnel syndrome observed in 9.5 % of diffuse and 5.3 % of limited subset, tendon friction rub in 14.3 % of diffuse and in 5.1 % of limited patients, prevalence of muscle weakness and elevated CPK in patients with dcSSc was about twice compared to lcSSC patients. Isolated PAH was observed only in patients in lcSSc subset. In patients with dcSSc subset, 25.0 % had pericarditis compared to 8.6 % in lcSSc subset.

ANA, ANA pattern and correlation with disease subsets

Out of all 100 patients who underwent antibody assay, 93 had positive ANA. The duration from first symptom to the time of entry to the study in ANA positive and ANA negative patients were 73.7 and 42.3 months, respectively, ($p = 0.30$).

In patients with positive ANA significantly more frequent skin score, Raynaud's phenomenon and digital ulcer/gangrene were observed ($p < 0.05$). All ANA negative patients were female with less severe skin involvement. One patient had a diffuse cutaneous type of disease. Out of 7 ANA negative sera, only in 4, PM/Scl, U1-RNP and TOPO antibodies were detected by line immunoassay technique.

Correlation of ANA pattern and specific auto-antibodies

ANA patterns were reported as speckled, centromere and nucleoli pattern in 85, 6 and 4 patients, respectively. Centromere pattern in immunofluorescent microscopy showed correlation with anti-centromere antibodies checked by line immunoassay technique ($r = 0.75$, $p = 0.001$). Speckled pattern was correlated with anti-TOPO ($r = 0.53$, $p = 0.001$) and U1 ($r = -0.434$, $p = 0.001$). No correlation was observed between nucleolar pattern in IFA microscopy and PM/Scl, U3-RNP and Th/To.

Concomitant presence of antibodies with TOPO

Out of 71 anti-TOPO positive patients, 3 (4.2 %) were also ACA positive, 8 (11.3 %) were Pol 3 positive, 21 (29.5 %) were PM/Scl positive, 4 (4.3) were Th/To positive and finally 1 % were U3-RNP and U1-RNP positive concomitantly.

Specific auto-antibodies in systemic sclerosis

Basic and demographic features did not show any significant differences within each auto-antibody groups

Table 2 Baseline, demographic and clinical features in two subtypes of Scleroderma

	Missing data	All patients	Diffuse	Limited	*p* value
Female no. (%)	0	87	18 (85.7)	69 (87.3)	1.00
Age at first symptom/years mean (SD)	0	36.5 (13.2)	32.4 (12.9)	37.6 (13.2)	0.10
Age at time of entry/years mean (SD)	0	41.9 (13.9)	38.8 (14.9)	42.9 (13.5)	0.15
Time interval from first symptom to first visit/months	0	67.5 (73.4)	67.8 (72.1)	67.4 (74.1)	0.98
Follow-up period from time of first symptom/months mean (SD)	0	131.4 (124.5)	115.7(122.6)	135.1 (125.5)	0.50
mRSS (median)[a]	0	10	**22**	**8**	**0**
Vascular no. (%)					
History of Raynaud's phenomena	0	95	#20 (95.2)	75 (94.9)	1.00
Periungal erythema	0	51	9 (42.9)	42 (53.2)	0.46
Periungal infarct	0	11	2 (9.5)	9 (11.4)	1.00
Digital tip pitting ulcer	0	55	12 (57.1)	43 (54.4)	1.00
Digital ulcer/gangrene	0	39	9 (42.9)	30 (38.0)	0.80
Digital amputation	0	5	1 (4.8)	4 (5.1)	1.00
Telangiectasia (hand/face/lip)	0	83	17 (81.0)	66 (83.5)	0.70
Fat pad atrophy	0	46	8 (38.1)	38 (48.1)	0.46
Raynaud seen by physician	0	65	11 (52.4)	54 (68.4)	0.20
Joint					
Arthritis > one joints	2	7 (7.1)	2/20 (10.0)	5/78 (9.3)	0.63
Carpal tunnel syndrome	0	6	2/21 (9.5)	4/79 (5.3)	0.60
Tendon friction rub	0	12	3 (14.3)	8 (10.5)	0.70
Muscle					
Muscle weakness	0	7	3/21 (14.3)	4/76 (5.1)	0.16
CPK > 2 normal	17	7 (8.4)	2/15 (13.3)	5/66 (7.4)	0.20
Gastrointestinal					
Esophageal	0	82	16 (76.2)	66 (83.5)	0.52
Diarrhea	0	14	4 (19.0)	10 (12.7)	0.48
Constipation	0	10	3 (14.3)	7 (8.9)	0.43
Pulmonary					
Fibrosis	23	32 (41.6)	5/13 (38.5)	27/64 (42.2)	1.00
Ground glass	23	13 (16.9)	3/13 (23.1)	10/64 (15.6)	0.68
FEV1/FVC <70 %	15	7 (8.2)	1/17 (5.9)	6/68 (8.8)	1.00
DLCO <70 %	19	39 (48.1)	11/17 (64.7)	28/64 (43.8)	0.17
PAP >45	28	7 (9.5)	1/16 (6.3)	6/57 (10.5)	1.00
Isolated PAH	28	3 (4.2)	0/15	3/58 (5.2)	1.00
Cardiac involvement					
Pericarditis	26	9 (12.2)	4/16 (25.0)	5/58 (8.6)	0.95
Symptomatic left ventricular heart failure	26	2 (2.7)	0/16	2/58 (3.4)	1.00
Arrhythmia requiring treatment	26	1 (1.4)	0/16	1/58 (1.7)	1.00
Pacemaker	26	2 (2.7)	0/16	2/58 (3.4)	1.00
Renal					
Blood pressure >139/89	6	16 (17.0)	1/16 (6.3)	15/78 (19.2)	0.20
Proteinuria >+1/hematuria/RBC cast	6	6 (6.4)	2/16 (9.5)	4/78 (5.1)	0.27

Bold numbers are indicative of statistically significant difference $p < 0.05$

[a] m*RSS* modified Rodnan Skin Score

regardless of positive or negative result as shown in Table 3.

The frequency of ACA in this study was 7 %, although ACA was exclusively positive in the lcSSc group, the frequency of ACA within the lcSSc subset was 8.9 % (Table 3).

There was significant difference in prevalence of anti-TOPO in dcSSc and lcSSc subset of disease, 90.5 versus

Table 3 Auto-antibodies prevalence and comparison in different clinical or paraclinical features

Antibody	Missing data	ANA+ No. = 93	ACA+ No. = 7	TOPO+ No. = 71	Pol3+ No. = 10	U1+ No. = 7	ANoA+ No. = 34
Presenting as dcSSc	0	21.50 %	0	26.80 %	20 %	0	14.70 %
Presenting as lcSSc	0	78.50 %	100 %	73.20 %	80 %	100 %	85.30 %
Female	0	86.00 %	85.70 %	85.90 %	90 %	100 %	79.40 %
Age (years) at time of Entry mean (SD)	0	**42.5 (14.1)**	45.7 (18.1)	42.1 (13.6)	49.0 (16.1)	45.3 (11.9)	41.6 (15.4)
mRSS mean (SD)[a]	0	**12.4 (8.0)**	8.4 (7.2)	**13.5 (8.3)**	12.8 (6.3)	6.4 (5.1)	11.9 (7.2)
Raynaud	0	**96.80 %**	85.70 %	95.80 %	100 %	85.70 %	97.10 %
Dig pitting ulcer	0	57.00 %	42.90 %	**62.00 %**	50.00 %	28.60 %	50.00 %
Dig ulcer/gangrene	0	**41.90 %**	42.90 %	43.70 %	60.00 %	14.30 %	41.20 %
Esophageal reflux	0	83.90 %	85.70 %	58 (81.7)	90.00 %	85.70 %	85.50 %
Diarrhea	0	12.90 %	0	10 (14.1)	10.00 %	14.35	17.60 %
Tendon friction rub	0	10.80 %	0	11.30 %	10.00 %	85.70 %	8.80 %
Muscle weakness	0	6.50 %	0	7.00 %	10.00 %	14.30 %	2.90 %
Arthritis > one joint	2	7.50 %	0	7.10 %	10.00 %	0	3.30 %
CPK elevation >2	17	8.90 %	0	8.80 %	22.20 %	0	10.00 %
Fibrosis in HRCT	23	43.20 %	20.00 %	**49.10 %**	**55.60 %**	0	**48.10 %**
PAH without fibrosis	28	2.90 %	0	0	0	20 %	**4.30 %**
FEV1 <70	15	8.60 %	20.00 %	**9.40 %**	**20.00 %**	0	**10.00 %**
DLCO <70 %	19	50.00 %	33.30 %	**55.00 %**	**33.30 %**	0	**43.30 %**
Pericarditis	26	12.70 %	0	12.20 %	0	0	4.20 %
Symptomatic left ventricular failure	26	2.80 %	0	2.00 %	0	0	4.20 %
Hypertension	6	17 %	33.30 %	**15.20 %**	**30.00 %**	14.30 %	21.90 %

Bold numbers are indicative of statistically significant difference versus comparative group $p < 0.05$

[a] m*RSS* modified Rodnan Skin Score

65.5 % ($p = 0.02$). In TOPO positive patients, mean prevalence of skin score, digital pitting ulcer, ILD, fibrosis and DLCo was significantly more frequent compared to negative TOPO patients; 8.3 versus 5.2 % ($p = 0.02$), 62 versus 37.9 % ($p = 0.02$), ILD in 70.2 versus 25.0 % ($p = 0.001$), 49.1 versus 20.0 % ($p = 0.02$) and 55.0 versus 28.6 % ($p = 0.03$), respectively.

In our study, anti-U1RNP was negatively associated with the presence of pulmonary fibrosis ($p = 0.03$) and interstitial lung disease ($p = 0.04$) (Table 3).

ANoA include antibody against PM/Scl, Th/To, U3-RNP and RNA Polymerase I, II, and III did not show any significant differences in two subset of disease or with specific clinical features.

Antibodies such as Th/To, U3 had very low prevalence; therefore, we excluded them from the study result. However, in analysis of ANoA antibodies, we looked into correlation of clinical features with the group of Th/To, U3 and PM/Scl antibodies.

Disease stages and auto-antibodies

Prevalence of auto-antibodies reactivity did not show differences in the early and late stages in the two subsets of disease ($p = 0.09$).

Discussion

In this cohort study, 79 % of individuals were classified as lcSSc and 21 % as dcSSc with a ratio of lcSSc to dcSSC subset of 3.8/1. This pattern also demonstrated in EUSTAR study where lcSSc subset of disease was more common than dcSSc with ratio of 1.6/1 [3].

The most frequent demographic and clinical features were middle-age females with lcSSc subset of SSc. This finding was partly demonstrated by Ferri et al. [11].

The female/male ratio in this cohort was 6.7/1 which is close to reported ratio in large EUSTAR cohort of 6/1 [3]. Different sex ratios were reported from UK 3/1 and Japan 14/1 [12].

We did not find any correlation between positivity of auto-antibodies and gender in contrast to other studies that demonstrated difference in ACA prevalence based on gender [2, 3, 13].

Comparable to EUSTAR cohort, our analysis showed no differences in the mean age of patients with different disease subsets.

Clinical findings in disease subsets

Clinical findings in the two disease subsets did not have any statistically significant difference except for skin score which was more frequent in dcSSc.

Contrary to our result, two previous studies (EUSTAR and Italian) [2, 11] demonstrated a significant difference in prevalence of arthritis in the two subsets. In our study, however, we found more of esophageal involvement compared to the EUSTAR and Italian. This might be related to the difference in inclusion criteria [3, 11]. We demonstrated that prevalence of heart involvement was numerically more in dcSSc subset compare to lcSSc form. This difference did not reach statistical significance. Our results are similar to the findings in large Italian study [11].

We found that disease stages at the entry (early or late) had no effect on the prevalence of auto-antibody positivity. This could be an indication that auto-antibodies could exist before any clinical manifestation of the disease is present and could remain there throughout the course of the disease [4].

Disease presentation according to auto-antibodies

In a recent meta-analysis, detection of ANA by IIF method showed an overall diagnostic sensitivity of 85 % and specificity of 54 % [14]. In our study, sensitivity of ANA was 93 %.

In sera of 7 % of our patients, no ANA was detected. In the other studies, prevalence of ANA negativity in SSc reported as 5–16 % [2, 11, 15].

We detected anti-TOPO positive antibodies in sera of 71 % of all patients and in sera of 90.5 % of dcSSc patients. Patients with lcSSc had high prevalence of TOPO (65.8 %) and low prevalence of ACA (7 %). However, ACA was found almost exclusively (100 %) in lcSSc patients.

Clinical features, frequency of scleroderma subsets and auto-antibodies prevalence vary in different countries and ethnics groups [2]. In French patients with SSc, anti-TOPO was reported positive in 38 % of those with dcSSc, and in Thiland where 100 % of patients had dcSSc, TOPO antibody was present in 76 % of patients. Prevalence of ACA is reported between 20 and 30 % [2, 10, 14, 16].

In an Australian study, most of their patients reported to have lcSSc subset with ratio of 6–1 (lcSSc/dcSSc). More patients in this study were ACA positive (50 %) compare to the report from Thailand [17].

These reports support the importance of genetic/ethnic background in SSc and might explain differences in auto-antibodies prevalences, especially TOPO and ACA in different countries. This may as well explains clinical differences in the two disease subsets.

High frequency of anti-TOPO antibodies in patients with lcSSc subset may be a reason for insignificant difference in pulmonary fibrosis in the two disease subset in our patients.

We reported more frequent ILD, fibrosis and DLco <70 in the TOPO positive patients. This result was similar to the other studies that showed anti-TOPO antibodies correlate with the presence and severity of radiographic interstitial pulmonary fibrosis [13, 18] and with a higher rate of decline in pulmonary function test [19].

The serologic test for AnoA was positive in 32 % of all patients; in 84.4 % of patients with lcSSc and in 15.6 % of patients with dcSSc. Positive ANoA was reported in 15–40 % of patients with SSc [19, 20].

Prevalence of anti-Th/To in our patients was 4 %, whereas in United States, it was reported 5 % and in Danish 2.2 % [2, 13]. We found PAH without fibrosis only in 5.2 % of the patients in lcSSc, which was less prevalent than the other series [2, 15]. Primary PAH is seen more prevalent in patients who have ACA, U3-RNP and Th/To antibodies [21]. Low prevalence of PAH in our patients might be due to low prevalence of these auto-antibodies.

We found the prevalence of 1 % for anti-U3 with no difference in clinical features between the two subsets. In previous researches, prevalence of anti-U3 has been reported between 4 and 6 % [2, 22, 23]. Anti-U3 RNP reported to be more frequent in men with significantly less joint involvement [24].

The frequency of anti-PM/Scl apparently varies across different ethnic groups. In Iranian patient, anti-PM/Scl found to be positive in 21 % of patients with SSc. In a study by Steen VD et al. [2], this was reported 2.5 %, and in Japanese patients with SSc, researchers could not detect anti-PM/Scl antibody [25].

Despite our results which did not show any association between PM/Scl and overlap syndrome or lcSSc subset, previous studies reported such correlations [25–27].

The prevalence of anti-RNA polymerase III in our patients was 13 % similar to the prevalence reported in Japan (10.7 %) and in United Kingdom (12 %) [28, 29].

Prevalence of anti-RNA polymerase III in North America is reported 10–23 % [25, 30].

We did not show any association between presence of anti-RNA polymerase III and dcSSc but we found that anti-RNA polymerase III antibody was more frequently present in our female patients (84.6 %). Our result is similar to what was reported by Nihtyanova et al. [31] in 2009.

None of our patients developed scleroderma renal crisis throughout the course of the study. This seems to be related to prevalence of more patients with lcSSc subset and presence of more frequent anti-TOPO antibodies in our patients. Previous reports demonstrated that renal crisis was low in Italy, Japan and Greece compared to the other reports from United States and northern Europe [1, 11–13, 32]. Genetic factors might partly explain this difference.

Association of anti-U1 RNP with overlap syndrome and lcSSc has been reported [33]. Other investigators found a

prevalence of about 6.5–10 % of anti-U1 RNP [13, 25] in SSc which was more frequent in lcSSc [25, 29]. However, we could not show association between anti-U1 RNP and any of the two disease subsets; lcSSc or dcSSc. We detected anti-U1 RNP antibody in only seven patients (7 %) who were all in lcSSc subset. It had no association with joint manifestations and negative association with the presence of pulmonary fibrosis ($p = 0.03$) and interstitial lung disease ($p = 0.04$). Our result was in contrast to the study of Ihn et al. where anti-U1RNP was closely correlated with the presence of pulmonary fibrosis and joint involvement [34].

Concomitant presence of TOPO with other auto-antibodies

It is claimed that ACA and anti-TOPO antibodies are always mutually exclusive [1, 35] and present in less than 1 % of SSc patients' serum simultaneously [36]. In our study, concomitant presence of ACA and anti-TOPO was detected in serum of 4.3 % of the patients (in 3 out of 71 TOPO positive patients). All those 4 patients who had positive anti-Th/To also were concomitantly positive for some of other SSc-related antibodies. We detected both anti-TOPO and anti-RNA polymerase III antibodies in serum of 11 patients.

Previous reports have demonstrated presence of anti-Th/To with TOPO, ACA and U1-RNP [37]. Presence of anti-TOPO and anti-RNP polymerase as mutually exclusive antibodies in dcSSc has been reported [31].

We observed that disease stages at the entry (early or late) had no effect on prevalence of the auto-antibody positivity. This could be an indication that auto-antibodies could be present at disease onset and persist throughout the course of disease regardless of the disease being in early or late stages and could continuously remain in the serum throughout the course of the disease [4].

Conclusion

We did not find any association between gender and presence of auto-antibody. In our patients, anti-TOPO antibody had a high prevalence in contrast to low prevalence of ACA antibody.

There were no differences in clinical features of the two disease subsets in patients with positive anti-TOPO and positive ACA. In patients with positive anti-TOPO antibody, we found association with higher skin score, vascular and pulmonary features of the disease. In patients with positive anti-U1 RNP antibody, no association with joint manifestation was found; however, we demonstrated negative association with the presence of fibrosis. There was no renal crisis in any of studies groups.

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Conflict of interest None.

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