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Correlation between autoantibodies and internal organs involvement in Iranian systemic sclerosis patients

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• Introduction

• Systemic sclerosis (SSc) is a rare-orphan connective tissue disease, classically characterized by the tightness of the skin. It is not necessarily limited to the skin and can involve other organs as well. The clinical picture of the disease based on the extent of skin involvement is generally divided into limited and diffuse SSc forms according to extend of the skin involvement (1). The researchers reported the association between serum autoantibodies and the subtypes of the disease in 1988, for the first time. It brings up the importance of these autoantibodies for the assessment of SSC. (1). Thereafter, new autoantibodies have been described in SSc that a number of which are shown to have clinical relevance (2). The combined clinical-serologic view to SSc and 2013 American College of Rheumatology (ACR) / European League Against Rheumatism (EULAR) classification criteria for SSc considerably helps physicians detect patients in the early stages of the diseases with high sensitivity and specificity (2-4). Evidence provides that 75 - 95% of SSc patients have a positive test for autoantibodies, specific or strongly associated with SSc.(2). Three phases are usually seen throughout the course of this disease; 1. Autoimmunity and inflammation 2. Functional and structural changes in small blood vessels and 3. Widespread fibrosis of the skin and different internal organs (3). Clinical cues pointing at SSc are subtle and few at early stages. Therefore, autoantibodies are considered as means for an early diagnosis (or suspicion) of SSc. Additionally, evidence shows that patients who test positive for these autoantibodies in the early stages of the disease, usually stay antibody-positive over the course of the disease (4). Different studies have shown an association between certain autoantibodies and clinical manifestations such as hand deformity, Raynaud's phenomenon, pulmonary fibrosis, overlaps, and an overall severity rate of the disease (5, 6). Various studies have previously shown that the distribution of SSc-related autoantibodies and their clinical correlations vary across the populations living in different geographical areas (6, 7). This makes it essential to assess patients' serologic profiles in view of their genetic background for a more practical and personalized clinical perspective towards each patient.

• Objectives

• This study aims to evaluate the correlation between various SSc-related autoantibodies and organ involvements among Iranian SSc patients.

Patients and Methods

• **Study design**

This is a retrospective cohort study of patients with systemic sclerosis diagnosed according to the 2013 American College of Rheumatology (ACR) /European League Against Rheumatism (EULAR) classification criteria. All eligible patients with SSc followed in the scleroderma clinic of Al- Zahra hospital in Isfahan (Isfahan province, Iran) from March 2012 to December 2020 were enrolled in the study.

• **Inclusion and exclusion criteria**

We included SSc patients with at least four years since their diagnosis. The exclusion criteria were smoking, malignancy, overlap syndrome, and irregular follow-ups.

• **Data collection**

A rheumatologist visited the patients in the special clinic of scleroderma in Al-Zahra hospital (the biggest referral hospital in the central region of Iran) and we reviewed the charts for clinical and para-clinical data. The history of clinical course, organs involvement, and para-clinical tests showing the state of organ function (e.g., echocardiography, high-resolution CT, and endoscopy) were recorded. We defined organs involvement based on the details presented in Table 1.

• **Serology**

The plasma was collected from blood sample of each patient stored in a -18 ℃ degrees centigrade freezer. The scleroderma autoimmune profile kit from EUROIMMUN Corporation© (via immunoblot method) was used for the detection of the specific autoantibodies. This kit is designed for detection of the following autoantibodies: Scl 70 (anti-topoisomerase-1), centromere antigen subunits (CENP-A and CENP-B), RNA Polymerase III subunits (RP11, RP155), fibrillarin, nucleolus-organizing regions (NOR 90), anti-Th/To, polymyositis PM/Scl 100, PM/Scl 75, Ku, platelet-derived growth factor receptor (PDGFR) and Ro-52. Details of the serologic testing show in Figure 1.

• **ical analysis**

• Descriptive statistics were reported as means and standard deviations for continuous variables and frequencies and percentages for categorical variables. The antibodies were recorded as dicotomus variables based on their positivity and were assessed for their correlation with clinical findings using Fisher's Exact test and two by two tables. A p-value equal to or less than 0.05 was considered significant. SPSS software version 19 was used for statistical analysis.

Table1. The definitions and measurement of organ involvement

Organ involvement	Indicators of involvement	Measurement /definition	Source of data
Cutaneous involvement	Skin Stiffness	Thickness and hardening of dermis(8)	Physical examination
	Microstomia	Defined as an ability of opening their mouth limited to the patient's three middle fingers(5)	Physical examination or documented in patients' records
	Raynaud's Phenomenon	A history of excessive cold sensitivity and recurrent events of sharply demarcated pallor and/or cyanosis of the skin of the digits(9)	Existence or a history of as expressed by the patients or documented in patients' records
	Telangiectasia	Erythematous matted skin lesions of vascular origin, thus they blanch after local pressure(9)	Found through physical examinations, formerly recorded in the patient's records
	Digital Ulcerations	Active ulcers were defined as denuded areas with defined borders and loss of epithelialization, loss of epidermis, and dermis distal to the proximal interphalangeal joint on the volar aspect of a finger(10)	Found through physical examinations, formerly recorded in the patient's records
	Calcinosis	Calcium deposition that cluster around joints(9)	Found by physical examination, or through radiologic study
MSK involvement:	Synovitis	Arthralgia, joint swelling, or morning stiffness(11)	By history, detected in physical examination, or a former patient's records
	Myopathy	Decrease proximal muscle power	Physical examination
	Tendon friction rub	The examiner to place the palmar aspect of his or her fingers across the tendon area being examined, and ask the patient to actively move the underlying joint through as full a range of motion as possible. A leathery, rubbing, "squeaking" sensation will be noted if a tendon friction rub is present.(12)	examination or recorded in the patient's records
	Digital Contracture	A distance of 1.9cms or more between the patient's palm and their fingertip at maximum flexion(5)	detected through examination or recorded formerly
Pulmonary involvement	Interstitial Lung Disease	Nonspecific interstitial pneumonitis (NSIP) Usual interstitial pneumonitis (UIP) Honeycombing and fibrosis	Seen on x-ray radiogram or high-resolution chest CT scan. A related pulmonary involvement confirmed by a pulmonologist.
	Pulmonary Fibrosis		
Cardiac involvement	Pulmonary Hypertension	Right atrial pressure of 40 $_{Water-mms}$ or higher(5)	transthoracic echocardiography
	Cardiomyopathy	An ejection-fraction of less than 45%, arrhythmias that require treatment, or complete heart block(5)	Electrocardiogram Echocardiography
	Pericarditis	Pericardial effusion	Confirmed by echocardiography
Renal involvement	Renal crisis	A sudden onset of hypertension (above 140/90 mmHg, a 30$_{mmHg}$ increase in systolic blood pressure, or a 20$_{mmHg}$ increase in diastolic blood pressure) and associated disorders, including an increase of more than 50% in serum creatinine or above 120% of normal range, proteinuria (above +2 in urine qualitative assessment and urine protein / creatinine ratio above normal range); Microscopic hematuria, thrombocytopenia, hemolysis, or hypertensive encephalopathy(13)	Existence or a history of as expressed by the patients or documented in patients' records
GI involvement	GE reflux disease	Heart burn and regurgitation or esophagitis	History expressed by the patient, recorded in their files, or confirmed by endoscopic studies
	Chronic Constipation	Fewer than three bowel movements a week more than three month (14)	
	Chronic diarrhea	Persistence alteration of stool consistency from the norm with loose stool and increase frequency of greater than 4 weeks duration(15)	

Results

• Forty-six patients including 40(87%) women and 6 (13%) men were enrolled in our study. Twenty-eight (58.3%) patients had the diffuse, and 18 (37.5%) patients had the limited type of scleroderma. The mean age of patients was 43±10.9, and the mean disease duration was 10.1±4.9 years. Raynaud phenomenon (100%) and gastroesophageal reflux (91.7%) were the most common, but renal crisis (2.1%) and pericardial effusion (6.3%) were the rarest forms of organ involvement in our study.

• The most commonly found autoantibodies were anti-topoisomerase-1 (77.1%) and anti-Th/To (33.4%), respectively. Table 4 shows the measured autoantibody frequencies and a comparison of the two clinical SSc subtypes. Anti-Scl 70 was significantly more prevalent in the diffuse SSC group (P= 0.011) and CENPA in the limited type of patients (p= 0.046). Other auto-antibodies did not differ significantly between the two types of systemic sclerosis. Anti-RP 11 was the least commonly detected autoantibody in our study, and aside from that, anti-PDGF (platelet-derived growth factor) was not detected in our participants. Results of comparative frequencies of autoantibodies among different clinical types of our SSc patients show in Table 2.

• Anti-topoisomerase-1 antibody showed an association with interstitial lung disease, tendon friction rub and cardiomyopathy. Autoantibodies NOR-90, anti-Th/To, PM/Scl 100, Ku and Ro 52 were associated with heart disease and Ro 52 with pericardial effusion. The autoantibodies to Scl 70, PM/Scl 75, PM/Scl 100, Ku, and Ro 52 were found significantly associated with tendon friction rub and anti-Th/To was associated with the presence of myopathy. CENPA was correlated with digital ulcers. We did not find any significant correlation between SSc-associated organs and the remaining autoantibodies tested in our study. Table 3 summarizes these correlations and predictive values of autoantibodies for organs involvements.

Table 2. Comparative frequencies of autoantibodies among patients with different clinical types.

Autoantibody	Limited N(%)	Diffuse N(%)	Total N(%)	p-value for between-type difference Fisher's exact test
Anti-topoisomerase I (Anti-Scl-70)	12(66.7)	28(100)	41(77.1)	0.011 (Cramer's V=0.492)
Anti-centromere A (CENPA)	5(27.8)	3(10.7)	8(16.7)	0.046 (Cramer's V=0.417)
Anti-centromere B (CENPB)	5(27.8)	3(10.7)	8(16.7)	1
Anti-RNA polymerase 11 (RP-11)	0(0)	2(7.1)	2(4.1)	1
Anti-RNA polymerase 155 (RP-155)	2(11.1)	7(25)	9(19.8)	0.4
Anti-U3RNP (fibrillarin)	1(5.6)	3(10.7)	3(6.25)	1
Anti- nucleolus organizer region-90 (Anti-NOR90)	1(5.6)	3(10.7)	4(8.33)	0.6
Anti-Th/To ribonucleoprotein (Anti-Th/To)	4(22.2)	13(46.4)	17(33.4)	0.3
Anti-PM/Scl-75	1(5.6)	4(14.3)	5(10.7)	0.3
Anti-PM/Scl-100	1(5.6)	3(10.7)	4(8.4)	0.6
Anti-Ku	3(16.7)	5(17.9)	8(16.4)	1
Anti-PDGFR	0	0	0	-
Anti-Ro-52	6(33.4)	6(20.4)	12(25)	1

Table 3. The correlation and predictive values of autoantibodies for organs involvement

Autoantibody	Organ involvement	Cramer's V(ϕc)	P value
SCL 70	ILD*	0.426	0.039
	Tendon friction rub	0.450	0.026
	Cardiomyopathy	0.485	0.013
CENPA	Ulcers	0.417	0.046
CENPB	None*	-	-
RP 11	None*	-	-
RP 155	None*	-	-
Fibrillarin	None*	-	-
NOR-90	Cardiomyopathy	0.433	0.034
Th/To	Myopathy	0.436	0.033
	Cardiomyopathy	0.479	0.015
PM/Scl 75	Tendon friction rub	0.435	0.008
PM/Scl 100	Tendon friction rub	0.475	0.006
	Cardiomyopathy	0.475	0.006
Ku	Tendon friction rub	0.444	0.011
	Cardiomyopathy	0.430	0.014
Ro 52	Tendon friction rub	0.490	0.026
	Pericardial effusion	0.475	0.034
	Cardiomyopathy	0.467	0.040

Discussion and Conclusion

Interestingly, all of our patients have at least one specific autoantibody whilst in other studies; the prevalence of these autoantibodies was less than ours (2). We found a correlation between anti-topoisomerase-1 antibodies and diffuse scleroderma as well as the development of interstitial lung disease and cardiac disease. Anti CENP-A correlated with limited scleroderma and digital ulcers. Anti-PM/Scl75, PM/Scl100, anti-Ku and anti-Ro52 autoantibodies were correlated with presence of tendon friction rub. Anti-Th/To was associated with a higher rate of myopathy. Cardiomyopathy was more common among patients with higher levels of NOR-90, anti-Th/To, PM/Scl100, anti-Ku and Ro52 autoantibodies. The latter was also associated with pericardial effusion.

Anti-centromere antibodies are widely accepted to be associated with limited cutaneous SSc, development of pulmonary hypertension, less severe course of the disease, and with a lower prevalence of lung fibrosis, cardiomyopathy, or renal involvement. We found an association between anti-CENP-A or CENP-B with digital ulcers; however, no association between anti-centromere antibodies and other involved organs detected. Likewise, Volpe et al (19) reported no significant association between the clinical severity of the illness and higher levels of anti-CENP-B. In a multicenter cohort of 802 SSc patients, Perosa et al also reported anti-CENP-A and CENP-B to be linked with a lower rate of clinical symptoms and lower clinical severity, since unlike what we found, pulmonary hypertension was more common in patients with positive anti-CENP (18).

Anti-PM/Scl antibodies were found to be associated with overlap syndromes in Germany, with myositis and ILD in Canada (2). The results from a cohort of 280 SSc patients showed that patients with a positive anti-PM/Scl have a higher likelihood of muscle involvement and lung fibrosis, but little GI involvement was seen (20). Other studies have also confirmed the correlation of PM/Scl antibodies with muscle involvement (4, 5). Both PM/Scl 75 and PM/Scl 100 have been associated with the tendons friction rub and cardiomyopathy in our studyIn a multi-national cohort consisting of SSc patients, anti-Ro52 was reported as the second most prevalent antibody among SSc patients and was strongly associated with ILD (22). Except for anti-topoisomerase-1, we did not find any significant correlation between the other autoantibodies and ILD. However, in our study, for the first time, anti-Ro 52 was observed to be associated with tendon friction rub, cardiomyopathy, and pericardial effusion. These differences may be due to geographical differences and various genetic backgrounds of our patients.

Anti-NOR90 exists in several rheumatic diseases, including SSc, but it is a rare autoantibody (23). In a Japanese study on anti-NOR90 among autoimmune rheumatic diseases, only nine patients out of 91 included subjects were positive for this autoantibody, only three of them had SSc (24). As well, another study reported, "no correlation" between anti-NOR90 and systemic sclerosis (25). This autoantibody has been postulated to be related to a mild internal organ involvement and the limited type of cutaneous SSc. In our study, the prevalence of anti-NOR90 was low among patients with limited scleroderma, but it was associated with cardiomyopathy. Anti-fibrillarin (U3 RNP) antibodies are significantly more prevalent in Americans of African origin, and men (23). Anti-U3 RNP was associated with overlap syndromes, myositis, joint involvement, and pulmonary hypertension in a former study (26). This autoantibody is associated with multi-organ involvement and is more frequent in the diffuse type of SSc with severe pulmonary involvement (2). We found no significant association between this autoantibody and clinical features of SSc. Although our study shows a higher rate of autoantibody among patients with diffuse disease, the overall prevalence of this autoantibody was low, and this difference was not significant.

Anti-RNA polymerase III antibodies have a highly varying prevalence among different studies; ranging from 0 to 41% (27). Geographical factors were suggested for this matter, but the variety is not well explained yet. A systematic review on over 8,000 patients shows an overall prevalence of 11 % (27). These autoantibodies are shown to be associated with severe skin thickening and the occurrence of renal crisis (27, 28). Our study showed a very low-frequency of these autoantibodies and their presence was not related to any clinical symptoms.

Anti-Ku is also a rare autoantibody, the prevalence of which range from 1.5 to 5% (5). In a cohort on 625 SSc patients, only 2.2% were positive for anti-Ku (29) and related to skeletal involvement (particularly myositis). Another study in Italy reported a prevalence of 2% (8/379) for anti-Ku in a cohort of 7239 patients with autoimmune disease(30). They also found association of the antibody with muscle involvements. In our study, the prevalence of anti-Ku was more than reported literature (21%), but no correlation found between anti-Ku and muscle involvement. However, we found a significant association of these autoantibodies with cardiomyopathy and tendon friction rub.

Our study shows that certain SSc-related autoantibodies are associated with specific clinical findings among Iranian SSc patients, while some of them differ from literature findings. It may be because of the different genetic backgrounds of our patients in this geographical region. Some of the mentioned autoantibodies in this study might predict organ involvement, especially in early diagnosed patients. These biomarkers may predict which patients are more likely to develop organ-specific damage and to make a diagnostic or therapeutic plan individually.

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