

## Anti-Annexin V Antibodies: Association with Vascular Involvement and Disease Outcome in Patients with Systemic Sclerosis

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### Abstract

**Background:** Systemic Sclerosis (SSc) is characterized by skin thickening, fibrosis and vascular obliteration. The onset and course are heterogeneous. Prominent features include autoimmunity, inflammation and vascular damage.

**Aim of study:** To measure the level of serum Anti-Annexin V antibodies in SSc patients and to study its significance in relation to vascular damage in these patients.

**Patients and methods:** Twenty patients with SSc (12 with diffuse SSc and 8 with the limited form) and 10 healthy age and sex matched volunteers as controls were all subjected to routine laboratory testing and immunological profiling including antinuclear, anti-Scl-70, anticentomere, anticardiolipin antibodies and anti-annexin V antibodies titres. Vascular damage was assessed by clinical examination and assessment of the disease activity score, nailfold capillaroscopy and colour flow Doppler of the renal arteries; Doppler echocardiography was used for assessing pulmonary hypertension.

**Results:** Anti-annexin V antibodies were detected in 75% of patients. Comparisons between anti-annexin V in diffuse and limited subgroups showed no significance; however a statistically significant positive correlation was found between Anti-annexin V titre and the degree of vascular damage in SSc patients. Anti-annexin V increased significantly in patients with severe vascular damage in comparison with those less affected ( $15.3 \pm 6.6$  vs.  $11.25 \pm 3.6$ ,  $P < 0.05$ ). A significant positive correlation was found between Anti-annexin V titre and both the ACL titre ( $r = 0.79$ ,  $P < 0.001$ ) and the resistive index of the main renal artery ( $r = 0.42$ ,  $P < 0.05$ ).

**Conclusion:** Anti-annexin V antibodies were significantly present in sera of patients with SSc. Patients with more severe forms of vascular damage had higher titres of these antibodies. Anti-annexin V antibodies are a sensitive predictor of vascular damage in SSc and could serve as a useful parameter in discriminating patients with a higher risk of vascular affection from those without.

**Keywords:** systemic sclerosis, anti-annexin V antibodies, vascular damage

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## Introduction

Systemic sclerosis (SSc), a generalized disorder of connective tissue, is characterized clinically by skin thickening, progressive fibrosis and vascular obliteration that mostly involves the skin, gastrointestinal tract, lungs, heart and kidneys. The onset and course of the disease is heterogeneous. The prominent features of the disease include autoimmunity, inflammation and vascular damage.<sup>1</sup>

Patients with SSc can be classified into two distinct clinical subsets, diffuse and limited, with different patterns of skin and internal organ involvement and autoantibody production. Raynaud's phenomenon (RP) is a hallmark of the disease. Skin induration and internal organ dysfunction are also common features. Pulmonary involvement is second in frequency only to esophageal involvement as a visceral complication of SSc. Interstitial lung disease and pulmonary vascular disease, particularly pulmonary arterial hypertension, are the most commonly encountered types of pulmonary involvement in SSc.<sup>2</sup>

Clinical evaluation and laboratory testing, along with pulmonary function tests, Doppler echocardiography and high-resolution computed tomography of the chest are useful diagnostic tools in establishing a diagnosis in SSc patients and detecting visceral involvement. Nail-fold capillaroscopy (NFC) represents the best method for analyzing microvascular damage in SSc.<sup>3</sup>

Annexin V, a member of the lipocortin family, is expressed within various cells, including nucleoli. Many of these proteins are target molecules for autoantibodies generated in connective tissue diseases, especially in scleroderma and overlap syndromes. Annexin V inhibits prothrombin activation and is able to prevent thrombus formation under normal venous and arterial blood flow conditions. Antibodies to Annexin V have been identified in association with several pathological conditions, such as fetal loss, and venous and/or arterial thrombosis in Systemic lupus erythematosus patients, as well as digital ischemia and gangrene in SSc; however, their true pathogenic role remains to be proven.<sup>4,5</sup>

## Aim of the Study

The aim of this study was to measure the level of serum Anti-Annexin V antibodies in patients with SSc and to study their significance in relation to vascular damage in these patients.

## Patients and Methods

This study was conducted on 20 patients, diagnosed with SSc according to the American College of Rheumatology classification criteria.<sup>6</sup> They were attending the rheumatology outpatient clinic and the inpatient departments of internal medicine and chest in Ain Shams University Hospital. Informed consents were obtained from the patients and the study was approved by the Ain Shams Medical ethics committee. Ten healthy age and sex matched volunteers were included as a control group. All patients and controls were subjected to the following full history taking and thorough clinical examination, which included an assessment of their SSc disease activity index score which included the identification of 11 independent variables predictive of disease activity in SSc:

- Severity of RP and severity of shortness of breath in the past week, both measured on a numerical rating scale ranging from 0–10.
- Average number of episodes of RP per week, in the past month.
- Worsening of scleroderma in the past month.
- Pulse rate per minute.
- Presence of basilar crackles.
- Modified Rodnan skin score ranging from 0–51.
- Swollen joint count ranging from 0–28.
- Weight loss in the past year.
- Hematocrit value.
- Forced vital capacity measured as percent predicted.<sup>7,8</sup>

## Laboratory investigations

Routine laboratory testing included a complete blood count, and erythrocyte sedimentation rate (ESR) and kidney function tests (blood urea nitrogen and serum creatinine). Immunological tests for antinuclear antibody (ANA) and anticentromere antibodies (ACA) was performed by the indirect immunofluorescence technique. AntiScl-70 (anti-topoisomerase antibodies) and anticardiolipin antibodies (ACA: IgG and IgM) were tested using the ELISA technique. The anti-annexin V antibodies titre used the ZYMUTEST anti-Annexin IgG ELISA kit. [Hyphen-BioMed, France.]: to measure the IgG isotype of auto-antibodies to annexin V in human serum.



## Test methodology

The ZYMUTEST anti-annexin V IgG ELISA uses a highly purified human recombinant annexin V for isolating the auto-antibody to annexin V. A diluted serum is added to an annexin V-coated microtest well. If autoantibodies to annexin V are present, they bind to the immobilized annexin V. Following washing, bound auto-antibodies of the IgG isotype are revealed with a goat anti-human-IgG (FC gamma specific) peroxidase conjugate. Following another washing step, the peroxidase substrate tetramethylbenzidine (TMB) is added to the microtest well in the presence of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and the subsequent enzymatic reaction yields a solution with a blue colour. Addition of sulphuric acid stops the reaction and turns the solution color to yellow. The intensity of the colour developed is directly proportional to the amount of IgG isotype anti-annexin V autoantibodies in the sample.

## Sample collection and storage

Blood samples were allowed to clot for 30 minutes at room temperature and centrifuged for 10 minutes at 5000 rpm. The serum was then collected in aliquots and stored at -20 °C.

## Interpretation of the results

Results are expressed according to the A<sub>450</sub> values obtained for samples and controls, using the calibration curve. Results are expressed in units relative to the calibrator (AU/mL) as follows: negative <10 AU/mL, 'grey zone' 10–20 AU/mL, low positive ≥20–<50AU/mL, moderate positive ≥50–<100AU/mL and high positive ≥100 AU/mL.

## Widefield nailfold capillaroscopy

Examination was done by means of a Leica Wild M3Z stereomicroscope (Leica AG, CH-9435 Heebrugg, Switzerland). All fingers were examined and the distal end of the capillaries was evaluated as regards morphology, devascularization and endothelial injury. NFC findings were classified into three patterns:

- **Early (E) pattern:** Few giant capillaries (2–3 per field), little capillary haemorrhage (2–3 capillary haemorrhages per field) and relatively well preserved capillary distribution.
- **Active (A) pattern:** Frequent giant capillaries (5–6 per field), frequent capillary haemorrhage

(>5 capillary haemorrhages per field), moderate loss of capillaries and avascular areas, and mildly disorganized capillary architecture.

- **Late (L) pattern:** Irregular enlargement of the capillaries (with few or no giant capillaries), absence of hemorrhage, severe loss of capillaries and large avascular areas (2–3 per field), frequent bushy capillaries and severe disorganization of the capillaries (4–5 per field).<sup>9</sup>

## Pulmonary function tests

Forced vital capacity (FVC), forced expiratory volume I (FEV<sub>1</sub>), FEV<sub>1</sub>/FVC% and forced mid-expiratory flow rate (FEF<sub>25–75</sub>) were estimated using an automated flowmeter for measuring spirometric function. Patients were categorized as follows:

- A restrictive pattern if the value of FVC as <75% of the predicted value
- An obstructive pattern if the value of FEV<sub>1</sub>/FVC% was <75% of the predicted value
- A small airway affection if the value of FEF<sub>25–75</sub> was <75% of the predicted value.

## Doppler echocardiography

This was performed to calculate the mean pulmonary artery pressure to assess pulmonary hypertension.

## Colour-flow doppler

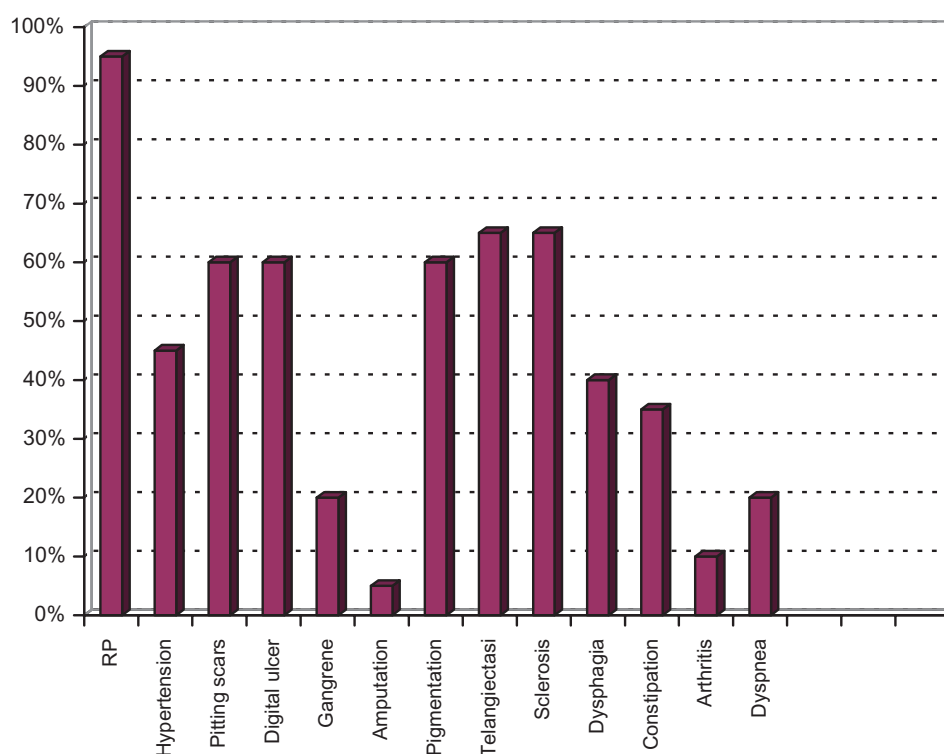
This method was used for examination of the renal arteries to detect any signs of luminal stenosis with calculation of the resistance index (RI). The RI was determined from the analysis of the spectral waveform as follows:

$$RI = \frac{SF_{peak} - DF_{min}}{SF_{peak}}$$

Where  $SF_{peak}$  is the peak systolic frequency shift and  $DF_{min}$  is the lowest diastolic frequency shift. The RI was calculated as a mean value obtained with six wave forms on the main renal artery and the interlobar and cortical vessels. All reported RI values were multiplied by a factor.

## Statistical methods

The clinical and laboratory data were transferred to IBM cards using an IBM personal computer with the statistical software package "Microstat Version 2" to obtain the following:



**Figure 1.** The frequency of the clinical data of the SSc patients.

- **Descriptive statistics:** Mean, standard deviation (SD), minimum and maximum values range, number and percentage of qualitative data
- **Analytical statistics:** Student’s t-test to compare between two independent means, Pearson’s correlation coefficient to find the relationship between different variables in the same group, and the Chi-square test to compare between different groups as regards qualitative data.

For all tests,  $P > 0.05$  was insignificant (NS),  $P < 0.05$  was significant (S), and  $P < 0.001$  was highly significant (HS). All data was graphically

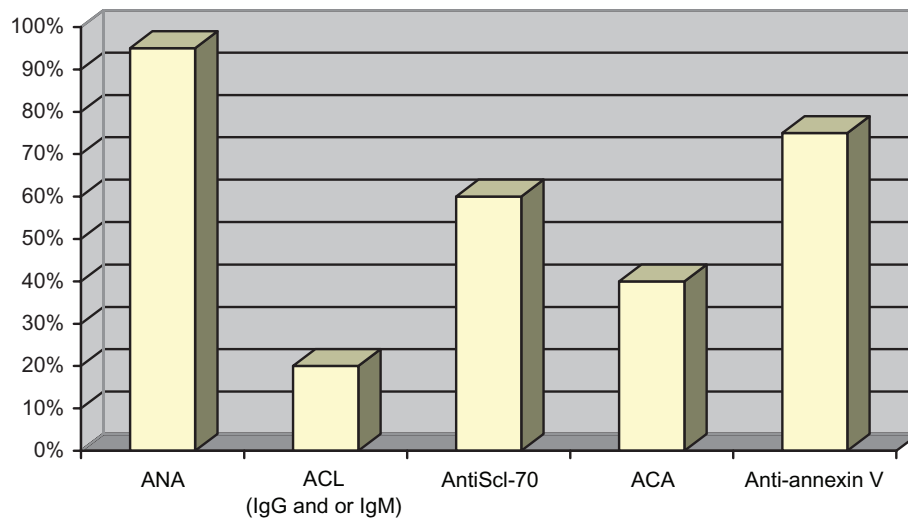
represented using Harvard graphics and Power Point programs.

### Results

This study was conducted on two groups: **Group I** included 20 female SSc patients, ages ranging from 28 to 56 years with a mean  $\pm$  SD of  $40.2 \pm 12.8$ . The disease duration ranged from 3 to 16 years with a mean  $\pm$  SD of  $6.7 \pm 4.12$ . **Group II** included 10 healthy female subjects as controls, ages ranging from 26 to 55 years with a mean  $\pm$  SD of  $41.4 \pm 11.17$ . For all patients, the parameters for assessing the disease activity index were estimated individually for each

**Table 1.** Comparison between group I and II as regards laboratory data.

Data	Group I Mean $\pm$ SD N = 20	Group II Mean $\pm$ SD N = 10	P	Significance
ESR mm/hr	27.3 $\pm$ 20.11	9.4 $\pm$ 4.07	<0.001	HS
Hb% gm/dL	11.18 $\pm$ 1.38	12.7 $\pm$ 0.44	>0.05	NS
RBCs $10^6/mm^3$	4.86 $\pm$ 0.5	5.1 $\pm$ 1.15	>0.05	NS
WBCs $10^3/mm^3$	8.21 $\pm$ 3.33	8.17 $\pm$ 1.56	>0.05	NS
Platlets $10^3/mm^3$	261.9 $\pm$ 21.13	211 $\pm$ 13.4	>0.05	NS
Blood urea nitrogen mg/dL	20.35 $\pm$ 8.53	20.1 $\pm$ 5.5	>0.05	NS
Creatinine mg/dL	0.79 $\pm$ 0.12	0.71 $\pm$ 0.10	>0.05	NS



**Figure 2.** Frequency of immunological markers in Group I.

patient. The disease activity index was estimated and a score was given for each patient.

Comparing the laboratory data between the patients and controls showed a highly significant difference in ESR ( $P < 0.001$ ), being higher in group I (Table 1).

The 20 patients were sub-divided into two groups according to AntiScl-70 and ACA antibody positivity. **Group Ia:** 12 patients (60%) had diffuse SSc (AntiScl-70 positive). **Group Ib:** 8 patients (40%) had limited SSc (ACA positive).

Anti-annexin V antibodies were present in 75% of patients (mean  $83.46 \pm 22.44$  AU/mL) vs. 0% in the controls (mean  $3.94 \pm 4.5$  AU/mL). Comparison between patients and controls as regards levels of anti-annexin V showed a highly significant difference ( $P < 0.001$ ). However, comparison between disease subtypes (Groups Ia and Ib) showed no statistical significance ( $P > 0.05$ ) (Tables 2 and 3).

Furthermore, the 20 SSc patients were re-classified into two groups according to the presence of severe

ischemic manifestations including digital ulcers, gangrene and amputation. **Group Is** included 11 patients (55%) with severe ischemic manifestations and **Group Im** included 9 patients (45%) without severe ischemic manifestations. Comparison of anti-annexin V antibodies between these groups revealed a statistically significant difference ( $P < 0.05$ ) (Table 4).

NFC was performed for all patients and controls. It showed five patients with the early pattern (25%), eight (40%) with the active pattern and seven (35%) with the late pattern. As regards the data of the controls, all showed an organized pattern. No abnormal capillary loops, capillary haemorrhage or avascular areas were detected. Anti-annexin V antibodies were negative in the five patients with the early pattern and positive in all patients with the active and late patterns. On comparing titres of anti-annexin V between patients with active and late patterns, no statistical significance could be detected ( $P > 0.05$ ) (Table 5).

**Table 2.** Comparison between anti-annexin V in Groups I and II.

Parameter	Group I Mean $\pm$ SD	Group II Mean $\pm$ SD	P	Significance
Anti-annexin V(AU/mL)	$83.46 \pm 22.44$	$3.94 \pm 4.5$	$<0.001$	HS

**Table 3.** Comparison between anti-annexin V in Groups Ia and Ib.

Parameter	Group Ia Mean $\pm$ SD	Group Ib Mean $\pm$ SD	P	Significance
Anti-annexin V(AU/mL)	$81.03 \pm 21.44$	$73.94 \pm 13.5$	$>0.05$	NS

**Table 4.** Comparison between Group Is and Im as regards anti-annexin V antibodies.

Parameter	Group Is Mean $\pm$ SD	Group Im Mean $\pm$ SD	P	Significance
Anti-annexin V(AU/mL)	82.69 $\pm$ 23.34	51.25 $\pm$ 23.65	<0.05	S

**Table 5.** Comparison of anti-annexin V between patients with active and late patterns.

Parameter	Active pattern Mean $\pm$ SD	Late pattern Mean $\pm$ SD	P	Significance
Anti-annexin V(AU/mL)	71.67 $\pm$ 23.9	83.01 $\pm$ 20.18	>0.05	NS

For all patients and controls, Doppler echocardiography was done to assess pulmonary artery pressure and to evaluate pulmonary hypertension. In the patient group, the pulmonary artery pressure ranged from 28 to 45 mmHg with a mean  $\pm$  SD of 35.00  $\pm$  6.00 mmHg. Pulmonary hypertension was detected in five patients (25%). Pulmonary hypertension was not detected in any of the controls and their pulmonary artery pressure ranged between 20 and 30 mmHg with a mean value of 21.00  $\pm$  5.00 mmHg. Comparing these results between patients and controls revealed a statistically significant difference ( $P < 0.05$ ) (Table 6).

Coloured flow Doppler (CFD) of main renal, interlobar and cortical arteries was done for all patients and controls, and the RI was calculated. Comparison between the patients and controls as regards RI values of main renal, interlobar and cortical arteries showed a statistically significant difference ( $P < 0.05$ ) (Table 7).

Correlation studies between anti-annexin V and various parameters of the disease were performed. There was a significant positive correlation with each disease activity score ( $r = 0.53$ ,  $P < 0.05$ ), ACL ( $r = 0.79$ ,  $P < 0.001$ ) and RI of the main renal artery ( $r = 0.42$ ,  $P < 0.05$ ) (Table 8).

A receiver—operator characteristic curve (ROC) was drawn and analyzed to study the sensitivity and specificity of the anti-annexin V titre with its (positive and negative) predictive value and efficacy in discriminating patients with vascular damage from those without. At a cut-off value of 50 AU/mL, the anti-annexin V titre can discriminate cases with vascular damage from those without, with 62.5% specificity, 91.7% sensitivity, a negative predictive value (NPV) of 83.3%, a positive predictive value (PPV) of 78.6% and an efficacy of 80%. **At the cut off value of 50 AU/mL:** true negative TN = 5, false negative FN = 1, false positive FP = 3, true positive TP = 11, specificity 62.5%, sensitivity 91.7%, NPV = 83.3%, PPV = 78.6% and efficacy = 80%.

## Discussion

Systemic sclerosis is an autoimmune connective tissue disease associated with vascular damage, which can occur early in the course of the disease resulting in decreased capillary blood flow. Clinically overt symptoms of SSc include RP and fingertip ulcers. In addition, various internal organs can be involved, predominantly the lung, the kidneys and the heart.<sup>10</sup>

**Table 6.** Comparison between Groups I and II as regards pulmonary artery pressure.

Parameter	Group I Mean $\pm$ SD	Group II Mean $\pm$ SD	P	Significance
Pulmonary artery pressure (mmHg)	35.00 $\pm$ 6.00	21.00 $\pm$ 5.00	<0.05	S

**Table 7.** Comparison between patients and controls regarding the RI of the main renal, interlobar and cortical arteries.

Arteries	Group I Mean $\pm$ SD	Group II Mean $\pm$ SD	P	Significance
RI Main renal artery	119.3 $\pm$ 32.4	52.33 $\pm$ 2.77	<0.001	HS
RI Interlobar artery	95.1 $\pm$ 32.8	51.71 $\pm$ 1.14	<0.05	S
RI Cortical artery	102.25 $\pm$ 31.2	50.4 $\pm$ 0.15	<0.05	S

**Table 8.** Correlation of anti-annexin V with variable disease parameters.

Variables	Anti-annexin V		
	r	P	S
Disease duration	0.25	>0.05	NS
ESR	0.13	>0.05	NS
Disease activity score	0.53	<0.05	S
ACL	0.79	<0.001	HS
RI of the main renal artery	0.42	<0.05	S
Pulmonary artery pressure	0.21	>0.05	NS

This study was conducted on two main groups. **Group I** included 20 female SSc patients, 12 with diffuse SSc (Group Ia) and 8 with limited SSc (Group Ib). Their ages ranged from 28 to 56 years with a mean  $\pm$  SD of  $40.2 \pm 12.8$ . The disease duration ranged from 3 to 16 years with a mean  $\pm$  SD of  $6.7 \pm 4.12$ . **Group II** included 10 healthy subjects as controls; all of them were females aged 26–55 years with a mean  $\pm$  SD of  $41.4 \pm 11.17$ .

In this study, RP was the presenting symptom in 95% of patients included in the study. RP is known to be the presenting symptom in 95%–100% of SSc patients. Dysphagia and arthralgia are also common symptoms in SSc. Digital ulcers and pitting scars were detected in up to 67% of SSc patients and telangiectasia was also frequently recorded.<sup>11,12</sup> ANA has been detected in 90% of SSc patients. AntiScl-70 and ACA are critical in differentiating both subtypes of SSc. ACL antibodies have been detected in SSc but with far less frequency than in other connective tissue disorders such as systemic lupus erythematosus and primary antiphospholipid syndrome. Recently, antifibrillin 1, anti-endothelial cells, anti-annexin V and anticollagen antibodies have been associated with SSc.<sup>13</sup>

In this study, anti-annexin V antibodies were present in 75% of the patients (Group I) and was not detected in any of the controls (Group II) (mean  $83.46 \pm 22.44$  vs.  $3.94 \pm 4.5$  AU/mL). Comparison between both groups showed a highly significant statistical difference ( $P < 0.001$ ). However, comparison of anti-annexin V antibodies in Groups Ia (diffuse) and Ib (limited) showed no statistical significance ( $P > 0.05$ ).

To study the relationship of anti-annexin V antibodies with digital ischemia, patients were re-classified

into two groups according to the presence of severe clinical manifestations of ischemia, including pitting scars, digital ulcers, gangrene and amputation. **Group Is** included 11 patients (55%) with severe ischemic manifestations; **Group Im** included 9 patients (45%) without severe ischemic manifestations. Comparison of anti-annexin V antibodies between these groups revealed a statistically significant difference ( $P < 0.05$ ). Furthermore, correlation of anti-annexin V titres with the disease activity score in the patient group showed a statistically significant positive correlation ( $r = 0.53$ ,  $P < 0.05$ ).

Similar studies detected anti-annexin V antibodies in 18.2% of SSc patients and 75% of the patients showed ischemic manifestations in association with anti-annexin V antibodies. This could be explained by the fact that digital ischemia may not simply be explained due to thrombosis and that anti-annexin V antibodies could possibly be related to the pathogenesis of digital ischemia in SSc patients.<sup>14</sup>

In addition, the anti-annexin V antibody titres in this study showed a highly significant positive correlation with ACL antibodies ( $r = 0.79$ ,  $P < 0.001$ ). Patients with antiphospholipid syndrome (APS) have been known to have a higher frequency of anti-annexin V antibodies, and thrombotic events have been reported more frequently in patients with positive anti-annexin V antibodies.<sup>15</sup> Furthermore, inhibition of annexin V binding to negatively charged phospholipids may be an additional pathogenic mechanism of APS.<sup>16</sup>

The patterns of SSc was first described by NFC in 1981 as enlargement of the capillary loops, loss of capillaries, disruption of the orderly appearance of the normal capillary bed and capillary haemorrhages.<sup>17</sup> NFC parameters were found to correlate with the diffuse form of SSc, the degree of cutaneous involvement and the presence of AntiScl-70 antibodies.<sup>18</sup>

Considering the SSc patterns observed in this study, 5% of patients had the early pattern, 75% had the active and 20% had the late pattern. Anti-annexin V antibodies were negative in the 5% of patients with the early pattern, and were positive in all patients with the active and late patterns. Comparison of anti-annexin V antibodies between patients with late and active patterns showed no statistical significance ( $P < 0.05$ ); however, the mean levels of anti-annexin V antibodies in patients with the late pattern were somewhat higher.



In this study, Doppler echocardiography was done to assess pulmonary artery pressure and to evaluate pulmonary hypertension. In Group I, the pulmonary artery pressure ranged from 28 to 45 mmHg with a mean  $\pm$  SD of  $35.00 \pm 6.00$  mmHg. Pulmonary hypertension was detected in five patients (25%). Pulmonary hypertension was not detected in any of the controls. Comparison between patients and controls revealed a significant difference as regards pulmonary artery pressure ( $P < 0.05$ ). However, correlation of anti-annexin V antibodies with mean pulmonary artery pressure in the patient group was of no statistical significance ( $P > 0.05$ ).

Both types of lung disease, namely pulmonary hypertension and restrictive lung disease, have been detected in SSc. Vasculopathy causing pulmonary hypertension typically occurs in SSc.<sup>19,20</sup> Although the results of echocardiography are accurate and adequate for diagnosing advanced pulmonary hypertension, it cannot be relied upon to exclude pulmonary hypertension or to be used as a screening test for early cases.<sup>21</sup> On the other hand, screening for pulmonary hypertension by Doppler echocardiography enables early detection of pulmonary hypertension at a mild stage.<sup>22</sup>

Considering the kidneys, comparison between the patients and controls as regards RI values of main renal, interlobar and cortical arteries showed a significant difference ( $P < 0.05$ ). RI values tended to decrease from the main renal artery to the cortical artery.

In similar studies, RI values of renal vessels have been found to increase significantly in SSc patients compared to controls, and significant reduction of RI values between the interlobar and the cortical arteries of patients has been reported, which indicates an increased resistance to distal flow to the interlobar artery.<sup>23</sup> Renal vascular damage studied by CFD in SSc patients that are clinically free of nephropathy has detected latent and subclinical renal damage in these patients.<sup>24</sup> Other studies measuring the RI of renal and interlobar arteries in SSc patients with and without signs of renal involvement detected a significant increase in RI values in patients with signs of renal involvement as compared to those without.<sup>25</sup> However, in contrast to this study, a recent study on 44 patients with End stage renal failure indicated that circulating levels of anti-annexin V microparticles were increased compared with 32 healthy subjects, as

were levels of microparticles derived from endothelial cells, platelets and erythrocytes. However, when arterial function was evaluated noninvasively in these patients, only endothelial microparticle levels correlated highly with loss of flow-mediated dilation and increased aortic pulse wave velocity.<sup>26</sup>

In this study, all patients had apparently normal kidney functions, yet the significant difference between RI values of patients and controls suggests the presence of subclinical renal involvement in SSc patients, which could be due to a vasculitic process, and may progress rapidly to SSc renal crisis or renal failure. This puts more stress on the importance of early detection of renal vascular impairment in SSc. In this study, a significant positive correlation was detected between the level of anti-annexin V antibodies and RI of the main renal artery ( $r = 0.42$ ,  $P < 0.05$ ), further suggesting the association of anti-annexin V antibodies with renovascular damage in SSc and thus highlighting the value of its early detection in the follow-up of scleroderma patients.

In conclusion, SSc is associated with microvascular and macrovascular organ damage, especially in the pulmonary and renal arteries. Anti-annexin V antibodies are detected in SSc, with increased expression in patients with ischemic manifestations. Anti-annexin V antibodies correlate positively with the disease activity score, ACL antibodies and RI of the main renal artery, and could serve as a sensitive predictor of vascular damage in SSc.

## Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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