Differential expression of α-smooth muscle actin and vascular endothelial growth factor in skin biopsy of patients with systemic sclerosis: a histological and immunohistochemical study

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Introduction

Systemic sclerosis (SSc) is a connective tissue disease characterized by three distinct pathologic processes: specific vascular changes, autoimmunity, and fibrosis [1].

Although a mild vasculitis may sometimes be present, the vascular pathology of SSc is not necessarily inflammatory and is best characterized as a vasculopathy. The vasculopathy associated with SSc is one of the major contributors to the clinical manifestations of the disease.

Background

Vasculopathy is a hallmark of systemic sclerosis (SSc). It contributes to many of its clinical manifestations and precedes fibrosis.

Objective

The aim of this study was to investigate the expression of α-smooth muscle actin (α-SMA) in skin biopsy of patients with SSc and correlate it with other manifestations of vasculopathy, including those seen on fundus fluorescein angiography and tissue vascular endothelial growth factor (VEGF) expression.

Patients and methods

This study included 25 patients with SSc and 10 healthy individuals. Patients underwent full history taking and a clinical examination. All participants underwent fundus fluorescein angiography. Skin biopsy was examined by H&E staining, Mallory triple staining, and immunohistochemical staining for α-SMA and VEGF.

Results

Histological examination showed loss of dermal papillae, hypovascularity of the dermis, and subepidermal fibrosis. Immunohistochemical staining of the vessel wall in skin biopsy samples showed a statistically highly significant increase in VEGF and a highly significant decrease in α-SMA in patients as compared with controls. There was a highly significant positive correlation between VEGF and duration of illness, Raynaud’s phenomenon, digital ulcers, disease activity score, and modified Rodnan Skin Score. As regards α-SMA, there was a highly significant negative correlation with Raynaud’s phenomenon, disease activity score, modified Rodnan Skin Score, and VEGF, whereas there was a significant negative correlation with digital ulcers. The strongest correlation (r) for the duration of illness was found with α-SMA, followed by VEGF.

Conclusion

α-SMA was found to be correlated to different manifestations of vasculopathy in SSc. It was found to be one of the early markers of vasculopathy among the other studied variables. Besides its diagnostic role in SSc vasculopathy, it could play a role in impaired vasculogenesis, making it a potential therapeutic target in the management of SSc.

Keywords:

α-smooth muscle actin, systemic sclerosis vasculopathy, VEGF

Microangiopathy might precede severe involvement of internal organs by many years [2].

The pathogenesis of SSc vasculopathy has been under investigation. It has been proposed that SSc vasculopathy involves vascular malformation and rarefaction. There is evidence for an insufficient angiogenic response in SSc. The dysregulation of angiogenesis leads to failure to replace damaged vessels (impaired vasculogenesis), resulting in a reduction in capillary density in patients with SSc [3].
The exact etiology and mechanism of this capillary rarefaction and impaired vasculogenesis is still under investigation.

α-Smooth muscle actin (α-SMA) is one of a number of proteins that are known to be involved in endothelial-mural interactions. Actin makes up the most abundant protein in many eukaryotic cell types. It polymerizes forming microfilaments that have an array of functions including regulating contractility, motility, cytokinesis, phagocytosis, adhesion, cell morphology, and providing structural support [4].

It is proposed that SSc vasculopathy is the result of an early event involving vascular injury and increased angiogenesis, but with defective vasculogenesis. The subsequent vascular malformation and rarefaction may be a function of angiogenic dysregulation, with overexpression of vascular endothelial growth factor (VEGF) but with a lack of proper interaction with smooth muscle cells needed to stabilize and organize blood vessels [5].

**Aim**

The present work aimed to study the expression of α-SMA in skin biopsy samples of patients with SSc and correlate it with other manifestations of vasculopathy including findings on fundus fluorescein angiography and tissue VEGF expression in order to investigate its role as a marker for vasculopathy and its possible pathogenic role.

**Patients and methods**

**Patients**

This study included 25 SSc patients (22 women and 3 men) who fulfilled the American College of Rheumatology classification criteria for SSc [6]. Their ages ranged from 38 to 52 years. They were selected from the Internal Medicine Department and Dermatology Outpatient Clinic of Ain Shams University Hospitals.

Ten age-matched and sex-matched healthy individuals were enrolled as controls.

Patients or controls with evidence of hypertension, diabetes mellitus, or cardiovascular events were excluded from the study.

**Methods**

All the patients were subjected to the following:

1. **Full history taking**: This included ascertainment of the duration of SSc, evaluation of Raynaud’s phenomenon, and assessment of symptoms of organ involvement.

2. **Clinical examination**: This included general, systemic, and musculoskeletal examination. Skin examination was performed and the extent of skin thickening in 17 body sites was graded according to the modified Rodnan Skin Score (mRSS) [7]. Digital ulcers, infares, gangrene, or telangiectasia were reported.

3. **Routine investigations**: This included CBC (Complete Blood Picture), ESR (Erythrocyte Sedimentation Rate), kidney function tests, chest radiograph, abdominal radiograph, ECG, echocardiography, pulmonary function tests, and upper endoscopy.

4. **Disease activity assessment**: A disease activity score for each patient was calculated according to a standard protocol including items for cutaneous and visceral involvement on the basis of data available from the clinical records (Table 1) [8].

5. **Fundus fluorescein angiography**: The fundus camera used was Top Con TRC-50 IA. The photos were acquired using a digital camera back Sony power head connected to a special PC and processed under special software for contrast, saving and printing: ImageNet for Windows-C-Multiformat Database. Fluorescein angiography was also performed to accurately demonstrate the changes in the retinal as well as choroidal vessels [9].

6. **Histological examination**: The skin specimens were fixed in 10% formalin and dehydrated, and paraffin blocks were prepared from which 5-µm-thick sections were cut and stained with H&E stain and Mallory triple stain for demonstration of collagen [10]. Other sections were cut on positive slides, which were deparaffinized using a series of xylene and rehydrated in descending grades of ethanol and rinsed in PBS. Immunoperoxidase staining of paraffin-embedded sections was then performed using the ChemMate Peroxidase/DAB System (Dako Cytomation, Hamburg, Germany). Monoclonal rabbit anti-human antibodies were purchased from Dako and the avidin–biotin complex technique was used.

**Avidin–biotin peroxidase immunohistochemical staining technique**

The paraffin sections were deparaffinized, hydrated, and placed in 10% H2O2 to block endogenous peroxidase activity. Unmasking of antigenic sites was carried out by transferring sections into a jar containing 0.001 mol/l citrate buffer (pH 6) and boiling in a microwave for 4 min at temperature grade VIII followed by 2 min at grade II. This was followed by blocking with serum blocking solution for 10 min. The specimens on the slides were then incubated with the primary antibody (1:500 monoclonal anti-VEGF and α-SMA protein) at room temperature for 2 h, and after washing they were incubated with biotinylated secondary antibodies (ABC Kit; 1:200). Freshly prepared diaminobenzidine was used as chromogen. Sections were incubated with diaminobenzidine for 10 min, then washed with tap water, counterstained with hematoxylin, dehydrated, and mounted. For negative control, the primary antibody was replaced by PBS [11].

**Statistical analysis**

Data were coded, entered, cleaned, and analyzed using the software SPSS (version 15; SPSS Inc., Chicago, Illinois, USA). For descriptive statistics, the median,
mean, and SD were used. Parametric (Student’s t-test) and nonparametric (Mann–Whitney U and Kruskal–Wallis) tests were used to compare between means. For studying the correlations between different variables, Pearson’s correlation and Spearman’s rank correlation coefficients were calculated. The level of statistical significance was set at P value less than 0.05 [12].

Results

This study included 25 SSc patients with a mean age of 31.9 ± 8.8 years; 22 were female and three were male. Ten healthy age-matched and sex-matched individuals were also included. Their mean age was 35.2 ± 5.4 years; eight were female and two were male. The mean duration of illness of the patients was 6.5 ± 5.8 years, with a minimum of 6 months and a maximum of 20 years.

Skin examination results
(1) The median value of the mRSS was 12 with a minimum score of 4 and a maximum of 27.
(2) Skin thickening affecting the face, upper extremities, and lower extremities was found in 15 (60%) patients, 20 (80%) patients, and five (20%) patients, respectively.
(3) Seven (28%) patients had fingertip ulcers, four (16%) had infarcts, and two (8%) had telangiectasia.
(4) The median value of the activity score was 12 with a minimum of 4 and a maximum of 17.
(5) Raynaud’s phenomenon was present in all patients.

The general clinical examination results
(1) Esophageal dysmotility was found in 15 (60%) patients, gastrointestinal reflux disease in 10 (40%), and colonic atony in two (8%).
(2) Pulmonary manifestations were detected in eight (32%) patients. Among those patients, the pulmonary function tests revealed a restrictive pattern in six (75%), obstructive pattern in two (25%), and small airway affection in four (50%) patients.
(3) Radiograph demonstrated bilateral diffuse reticulation in six (75%) patients.
(4) Renal involvement was present in five (20%) patients.
(5) M-mode echocardiogram revealed an increased right ventricular systolic pressure reflecting pulmonary hypertension in two (8%) patients.

Fundus examination results
Ten (40%) patients had fundus abnormalities, six (24%) had choroidal filling defects, and four (16%) had minute hyperfluorescence (Fig. 1).

Fundus fluorescein angiography revealed areas of hyperfluorescence and choroidal filling defects in patients with SSc (Fig. 2a and b).

Histological results
Sections of skin from control individuals stained with H&E demonstrated a stratified squamous keratinized epithelium of the epidermis and the underlying vascular connective tissue of the dermis with numerous dermal papillae (Fig. 3a). Mallory triple stain showed the collagen content of the dermis (Fig. 3b). The expression of VEGF was seen expressed in the keratinocytes of the epidermis and the endothelial cells of the blood vessels of the papillary and reticular dermis (Fig. 4a and b). α-SMA was expressed in the wall of the dermal vessels (Fig. 5a).

In patients with SSc, there was loss of the dermal papillae and hypovascularity of the dermis (Fig. 6a). Mallory triple stain revealed subepidermal fibrosis (Fig. 6b). There was an apparent marked increase in the intensity of VEGF expression in the blood vessels of the papillary and reticular dermis (Fig. 7a and b), and α-SMA expression was markedly decreased in the wall of the blood vessels (Figs 5b).

Morphometric and statistical results

Statistical analysis of the immunostained sections of the skin biopsy samples for VEGF showed a highly significant increase (P<0.001) in patients as compared with controls. A highly significant decrease in α-SMA staining (P<0.001) was seen in the wall of the vessels in skin biopsy specimens of SSc patients as compared with controls.

The Spearman rank correlation test showed a highly significant positive correlation (P<0.001) between VEGF and disease duration, Raynaud’s phenomenon, fingertip or toe ulcers, activity score of the disease, and mRSS.

As regards α-SMA, there was a highly significant negative correlation (P<0.001) with duration of illness, Raynaud’s phenomenon, mRSS, disease activity score, and VEGF, whereas there was a significant negative correlation with fingertip or toe ulcers (P<0.05) (Table 2).

The strongest correlation (r) of the duration of illness was found with α-SMA, followed by VEGF, on the Spearman correlation coefficient test. It was as follows in the order of strength: if α-SMA=0.86 (an inverse correlation as indicated by the negative sign) then VEGF=0.366 (Figs 8 and 9). Thus, shorter disease duration is statistically significantly correlated with a decrease in α-SMA staining, followed by increased VEGF staining.

Using the Kruskal–Wallis test we found a statistically significant difference (P<0.05) between patients with normal and those with abnormal fundus angiography regarding age, mRSS, activity score, and VEGF. A statistically highly significant difference (P<0.001) was found regarding the NFC (Nail Field Capillaryscopy) and α-SMA (Table 3).
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Figure 1. Fundus fluorescein angiography of controls (a, b) showing more or less normal results.

Figure 2. Fundus fluorescein angiography of patients with scleroderma systemic sclerosis showing areas of hyperfluorescence (a) and choroidal filling defect (b).

Figure 3. (a) Stratified squamous epithelium of the epidermis. Note the vascular connective tissue of the dermis with numerous dermal papillae. (b) The normal collagen content of the dermis.

H&E, control patient, (a) Mallory triple stain, (b) × 640.

Figure 4. (a) Expression of VEGF in the keratinocytes and endothelial cells of the papillary dermis (arrows). (b) Expression of VEGF in the endothelial cells of the reticular dermis (arrow).

Hematoxylin, control patients, × 640.
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Figure 5. (a) Expression of α-smooth muscle actin (α-SMA) in the wall of the blood vessels of the dermis of control patients. (b) Marked decrease in the expression of α-SMA in the wall of the blood vessels of the dermis of systemic sclerosis patients. Hematoxylin, × 250.

Figure 6. (a) Loss of the dermal papillae with hypovascularity of the dermis. (b) Marked increase in collagen content of the dermis. H&E, systemic sclerosis, (a) Mallory triple stain, (b) × 640.

Figure 7. (a) Marked increase in the reaction for VEGF in the endothelial cells of the papillary dermis (arrows). (b) Marked increase in the reaction for VEGF in the endothelial cells of the reticular dermis (arrows). Hematoxylin, systemic sclerosis, ×640.

Figure 8. Correlation between vascular endothelial growth factor (VEGF) and duration of illness in years.
Table 1. Indices for the evaluation of organ involvement in patients with systemic sclerosis

<table>
<thead>
<tr>
<th>Systems involved</th>
<th>Manifestations</th>
<th>Assigned value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Lc SSc</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Ic SSc</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Dc SSc</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Digital pitting scars or ulcers</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous calcinosis</td>
<td>1</td>
</tr>
<tr>
<td>Bone and joints</td>
<td>Acroosteolysis</td>
<td>1</td>
</tr>
<tr>
<td>Muscles</td>
<td>Weakness and/or atrophy</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Muscle enzyme elevation</td>
<td>1</td>
</tr>
<tr>
<td>Vessels</td>
<td>Thrombosis (any site)</td>
<td>2</td>
</tr>
<tr>
<td>Heart</td>
<td>Myocardial ischemia or necrosis (by ECG and/or scintigraphy)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Right ventricular hypertrophy</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Left ventricular hypertrophy</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Conduction defects</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Significant arrhythmias</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Pericarditis</td>
<td>1</td>
</tr>
<tr>
<td>Lungs</td>
<td>Dyspnea and/or cough and/or bibasilar rales</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Restrictive ventilatory defect, bibasilar fibrosis by radiography or CT scan</td>
<td>1</td>
</tr>
<tr>
<td>Kidneys</td>
<td>Creatinine clearance:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80–50 ml/min</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>50–20 ml/min</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&lt;20 ml/min</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Scleroderma renal crisis</td>
<td>3</td>
</tr>
<tr>
<td>Esophagus</td>
<td>Dysphagia</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Typical SSc dysmotility (by radiography, manometry, endoscopy)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Esophageal stricture</td>
<td>1</td>
</tr>
<tr>
<td>Small bowel</td>
<td>Malabsorption</td>
<td>2</td>
</tr>
<tr>
<td>Colon</td>
<td>Sacculation by radiograph</td>
<td>2</td>
</tr>
</tbody>
</table>

CT, computed tomography; SSc, systemic sclerosis.
Lc: limited cutaneous
Ic: intermediate cutaneous
Dc diffuse cutaneous

Table 2. Correlation between NFC, vascular endothelial growth factor, and α-smooth muscle actin with different variables

<table>
<thead>
<tr>
<th></th>
<th>Disease duration (years)</th>
<th>Raynaud’s</th>
<th>Ulcer</th>
<th>mRSS</th>
<th>Activity</th>
<th>VEGF</th>
<th>α-SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation coefficient (r)</td>
<td>NFC</td>
<td>0.710</td>
<td>0.575</td>
<td>0.842</td>
<td>0.838</td>
<td>0.823</td>
<td>−0.875</td>
</tr>
<tr>
<td>Significance (P)</td>
<td></td>
<td>0.000</td>
<td>0.003</td>
<td>0.000</td>
<td>0.000</td>
<td>0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>VEGF</td>
<td>0.366</td>
<td>0.863</td>
<td>0.863</td>
<td>0.638</td>
<td>0.696</td>
<td>−0.613</td>
</tr>
<tr>
<td>Significance (P)</td>
<td></td>
<td>0.006</td>
<td>0.600</td>
<td>0.000</td>
<td>0.000</td>
<td>0.006</td>
<td>0.001</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
<td>α-SMA</td>
<td>−0.860</td>
<td>−0.767</td>
<td>−0.480</td>
<td>−0.870</td>
<td>−0.878</td>
<td>−0.613</td>
</tr>
<tr>
<td>Significance (P)</td>
<td></td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

mRSS, modified Rodnan Skin Score; α-SMA, α-smooth muscle actin; VEGF, vascular endothelial growth factor.
Table 3. Level of significance of the comparison between patients with normal and abnormal fundus angiography regarding disease duration and activity score of the disease, modified Rodnan Skin Score, vascular endothelial growth factor, and α-smooth muscle actin

<table>
<thead>
<tr>
<th>Age</th>
<th>Disease duration/(years)</th>
<th>Activity</th>
<th>mRSS</th>
<th>VEGF</th>
<th>α-SMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>χ²</td>
<td>7.125</td>
<td>5.212</td>
<td>7.422</td>
<td>7.559</td>
<td>7.138</td>
</tr>
<tr>
<td>Significance</td>
<td>0.023</td>
<td>0.074</td>
<td>0.024</td>
<td>0.023</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Kruskal–Wallis test.
mRSS, modified Rodnan Skin Score; α-SMA, α-smooth muscle actin; VEGF, vascular endothelial growth factor.

Discussion

SSc represents a spectrum of connective tissue disorders characterized by chronic and debilitating fibrosis of the skin and internal organs. Ninety percent of patients exhibit chronic microvascular damage before the onset of clinical fibrosis. The vascular changes are a hallmark of the pathogenesis of SSc [13].

Angiogenesis is strongly disturbed in SSc. It is controlled by a subtle balance between endogenous stimulators and inhibitors. Angiogenic stimuli activate endothelial cells producing proteolytic enzymes that degrade the matrix. Endothelial cells proliferate and migrate to the perivascular area forming sprouts with subsequent lamination to form the capillary loops, followed by synthesis of new basement membrane and blood vessel maturation to form complete tubes through which blood can flow [14].

A large number of proangiogenic molecules have been defined, the most important of which is VEGF [15]. In this study VEGF was overexpressed in the vascular endothelial lining of affected skin of patients in comparison with controls. There was a highly significant correlation between VEGF and Raynaud’s phenomenon, fingertip ulcers, and fundus angiography, thus emphasizing its association with other manifestations of vasculopathy. This is in accordance with several studies on VEGF. It was found to be overexpressed in various types of skin cells of SSc patients. VEGF receptors were found to be upregulated in cells of SSc patients [15–17].

Although there is an increased expression of a large number of proangiogenic mediators including VEGF, there is a defective vasculogenesis in SSc. It has been shown that a brief upregulation of VEGF results in instability of newly formed vessels. In contrast, prolonged overexpression has deleterious effects because the vessels fuse in an uncontrolled manner and form a chaotic vessel network [18].

In accordance with this study Distler et al. [3] demonstrated overexpression of VEGF in patients with fingertip ulcers, noting the paradoxical effect of its prolonged overexpression in the formation of irregular vessels and defective vasculogenesis.

In this study, α-SMA was underexpressed in the lining of dermal blood vessels in skin biopsy samples of SSc patients. This was in accordance with a study by Dor et al. [19]. They noted a decreased expression of α-SMA staining with very few vessels in the dermis in SSc-involved skin. In SSc-uninvolved skin they detected malformed vessels that were still partially covered by pericytes. In healthy skin, α-SMA-positive cells in the wall of the vessels were clearly observed near the epidermis and in the deep dermis. Angiogenic dysregulation in SSc could be due to a lack of proper interactions with smooth muscle cells needed to stabilize and organize blood vessels resulting in decreased capillary density.

In this study α-SMA showed a statistically highly significant negative correlation with disease duration, Raynaud’s phenomenon, and VEGF staining. Moreover, it showed a statistically significant negative correlation with finger tip ulcers and infarcts. This emphasizes its relation to SSc vasculopathy. It was found that shorter disease duration was statistically significantly correlated with a decrease in α-SMA staining, followed by increased VEGF staining, implying earlier changes in α-SMA, followed by VEGF staining. It is advised, therefore, to take a skin biopsy to be stained immunohistochemically with α-SMA to confirm early diagnosis of SSc and also to detect early vasculopathy changes [20].

In accordance with our findings, Fleming et al. [21] studied SSc vasculopathy in skin biopsy specimens. They stated that SSc skin had true capillary rarefaction. Along with the loss of capillaries there was a dramatic decrease in vascular endothelial cadherin, a supposedly universal endothelial marker required for tube formation (lost in the scleroderma tissue).

Fundus angiography findings in studied patients were manifested as choroidal filling defects and minute areas of hyperfluorescence that point to a choroidal vasculopathy. It was detected in 40% of patients. Retinal vessels showed no abnormality. This is in accordance with the results of Serup et al. [22] who detected a choroidal vascular affection in 30% of their patients.

Choroidal vasculopathy in studied patients had a highly significant positive correlation with disease activity and a significant correlation with disease duration and VEGF. α-SMA staining showed a highly significant negative correlation with choroidal vasculopathy, further suggesting its relationship with SSc vasculopathy.

Conclusion and recommendation

α-SMA was found to be correlated with different manifestations of vasculopathy in SSc patients. It was
found to be one of the earliest markers of vasculopathy among the other studied variables. As SSc is a disease often recognized late in the course of the disease, early recognition will enable effective prevention and management of many of its serious irreversible visceral complications. Thus, besides its diagnostic role in SSc vasculopathy, it could play a role in the impaired vasculogenesis, which is the hallmark of SSc vasculopathy, making it a potential therapeutic target in the management of SSc.

**Acknowledgements**

Conflicts of interest

There are no conflicts of interest.

**References**


مقارنة اباني الألياف الأكتينية-الفا بداخل العضلات اللاإرادية و عامل النمو البطاني الوعائي في مرض التصلد الجلدي
دراسة هستولوجية و هستوكيميائية مناعية

 thuậnاء عادل لطفي و حنان عيسى حسن و غادة فاروق محمد و خالد حمدي محمود
قسم الجلدية - قسم الباطنة - قسم الهيستولوجيا - قسم الرمد كليه الطب
جامعة عين شمس

المقدمة: إصابة الأوعية الدموية في مرض التصلد الجلدي هي دور أساسي في ظهور أعراض المرض وتسبق حدوث التليف المصاحب للمرض.

الهدف من البحث: دراسة الألياف الأكتينية بداخل العضلات اللاإرادية في عينة من الجلد المصابة لمرض التصلد الجلدي ودراسة علاقة بعض الصور الأخرى لإصابة الأوعية الدموية منها تصوير الأوعية الدموية بشبكة العين بالصبغة و اباني عامل النمو البطاني الوعائي.

طرق البحث: اشتمل هذا البحث على نمط و قصر من مرضى التصلد الجلدي وعينة من الأشخاص الذين خضعوا لتصوير الأوعية الدموية بشبكة العين بالصبغة. تم أخذ عينة من الجلد من الأشخاص المشاركون في البحث من أجل دراسة التركيب النسيجي بالهيماتوكسيلين و الأيوسين و الصبغة الكيميائية المناعية لنسبة الألياف الاكتينية بداخل العضلات للا أرادية وعامل النمو البطاني الوعائي.

النتائج: لقد أظهرت الدراسة وجود فتح في خرائط الأوعية الدموية وتقسيم تحت الجلد. هذا مع وجود فرق بين مجموعة المرضى و مجموعة الضابطة من حيث نسبة الألياف الأكتينية بداخل العضلات اللاأرادية والعامل البطاني الوعائي.

لقد أظهرت الدراسة وجود علاقة ذات دلالة إحصائية بين حالة الانصهار بين عامل النمو البطاني الوعائي وظاهرة رينولد، القرح الطرفية للعمل، نسبة نشاط المرض، درجة تليف الجلد.

لقد أظهرت الدراسة وجود علاقة عكسيه ذات دلالة إحصائية بين نسبة الألياف الأكتينية حول الأوعية الدموية وظاهرة رينولد، نسبة نشاط المرض، درجة تليف الجلد و نسبة عامل النمو البطاني الوعائي.

لقد كانت هناك علاقة ذات دلالة إحصائية بين حالة انصهار بين حالة النمو البطاني الوعائي ونسبة النشاط أكثر قوة مع الألياف الاكتينية داخل العضلات اللاإرادية مما يشير إلى أهميتها في مراحل الامراض الأولى للمرض.

الاستنتاج: فئة نسبة الألياف الأكتينية حول الأوعية الدموية في عينة من الجلد لمرضى التصلد الجلدي تعتبر أحد الدلالات الأولى على وجود تأثير للشريعين الدموية.

الالياف الأكتينية بداخل العضلات اللاإرادية دور في حدوث تأثير للشريعين الدموية في هذا المرض مما يشير الي إمكانية استخدامها كهدف لدراسات علاجية مستقبلية.