Fingerprints evaluation in systemic sclerosis

Gheorghe Popa1*, Răzvan C. Ionitescu2, Romeo Potorac3, Carina Mihai1, Mihai Bojinca2, Victor Stoica2

Abstract: Systemic sclerosis (SSc), a connective tissue disease, involves vascular damage, autoimmunity and fibrosis. The aims of this study were: to identify a pattern of structural and biological changes of fingerprints (FP) in definite SSc patients and to establish it’s validity in time at the second and third patient visit after 2, respective 6 months from the first analysis. We also evaluated the degradation of FP constituents from the first visit during a month time. In all SSc fingerprints, pore density was lower, papillary combs were more flattened and papillary grooves narrower than in controls. Epithelial cells desquamation process was accelerated in SSc versus controls. DNA levels were statistically significant higher but their decrease over time was faster in SSc FP versus controls FP. On the second and the third visits, morphological constituents of FP were significantly deteriorated versus first visit suggesting accelerated skin degradation in evolution of SSc. Fingerprint evaluation is a non-invasive, simple, observational method and it may become the fundation of future attempt to find a fingerprint pattern capable to sustain the diagnosis of SSc in subjects with early scleroderma features.

Key words: sclerosis, vascular damage, fingerprint

Systemic sclerosis (SSc) is a connective tissue disease; it involves vascular damage, autoimmunity and fibrosis. The final aims of any SSc research are: to find a cure of the disease, which for the moment seems to be „a far away bridge”, and to distinguish the most suitable assembly of the parameters which can define „very early systemic sclerosis”. Regarding this second goal, our study encourages the research of FP in the future of very early SSc investigation tools. The main constituents of very early SSc picture proposed by experts in SSc at the “3rd EULAR / EUSTAR Educational Course, 2009, Paris” were: Raynaud syndrome, characteristic capillaroscopy pattern, presence of antinuclear antibodies (ANA), specific autoantibodies, scleredema turning into sclerodactily.

Our main objectives were: to identify a pattern of structural and biological changes of fingerprints (FP) in definite SSc patients and to establish its validity in time at the second and third patient visit after 2, respective 6 months from the first analysis.

Materials and Methods

Our study included 25 SSc patients (F/M, 22/3), mean age 50.2 ± 12.8 years. The study was carried out during 9 month period: August 2008 - April 2009. Disease duration from the first non-Raynaud symptom (DNR) was 9.6 ± 8.4 years; 14 patients had diffuse cutaneous SSc (dcSSc) and 11 patients had limited cutaneous SSc (lc SSc). We also enrolled 25 healthy sex-age matched subjects.

1*) Corresponding author; Prof. Univ. Ph D, Institutul de Studii pentru Ordine Publică, No.159-160 Olteniţei Street, Bucharest, Romania, Phone 0213324867, Fax 0213323855
2) Internal medicine and Rheumatology Unit, Dr. Ion Cantacuzino Hospital, Bucharest
3) DNA Departament, Forensic Institute, Bucharest, Romania
All subjects were examined in the same conditions and gave written consent for participation to this study. Ten patients were reevaluated for FP after 2 months and 9 of them were reexamined after 6 months from the first visit. We enrolled only the SSc patients which fulfilled the following CASE criteria:

- beginning of the disease more than 2 years.
- Raynaud phenomenon present
- ANA positive
- Antitopoisomerase I (SCL70) or Anticentromere (ACA) antibodies present
- SSc specific capillaroscopy pattern (Cutolo patterns).

Presence of minimum distal digital scars were considered mandatory for 10 patients.

We applied 4 methods to obtain FP: scanner, sampling on glass, sampling on paper and photography with professional device and also with stereomicroscope Olympus SZX7. The descriptive method, the method of laboratory morphological analyses, the method of physical chemical determination, and the method of reporting the degrading biochemical processes have been the methodological research basis for the investigated material.

The fingerprints were lifted on sterile glass. Lucia Forensic application was used for examining each fingerprint, allowing us to take over the images taken by the microscope video camera and by a digital photo camera, to process them at the same scale, to automatically make the necessary measures, to mark the specific elements, and to locate and orient two images and compare them. For the microscopic examination of FP we used a microscope with a 20x objective, having artificial lighting (white color filter, 70W). The microscopic preparation was done with Nuclear Fast Red (5g of Aluminiumsulphate), 0,1g Nuclear Fast Red, 100 ml distilled water.

We looked in a FP for: pore density, thickness of papillary combs, width of papillary grooves, epithelial cells microscopic disposal and DNA total quantification from FP- incorporated biological material. Quantifying the human DNA existing in fingerprints was carried out by using the 7500 Fast Real-Time PCR Systems and the following reactions: TaqMan Fast Univ PCR Master Mix, Human DNAQuant Assay, positive control, de-ionized water. POLILIGHT instrument was used for noticing FP, as well as a lighting source with a variable wave length between 420-460 nanometers, at an incidence angle of 45%.

We included all the subjects for the first step of our study, which was considered as moment ZERO. We now examined the characteristics from FP taken on glass from all the cases and controls. In this phase we used comparison method for the identification of the morphological differences between thickness of papillary combs and grooves, pores density and epithelial cells forms and microscopic disposal from SSc FP versus control FP. We measured the concentration of total DNA in picograms / microliter from SSC FP and controls FP. We also examined weakly DNA decrease levels in one month period from moment ZERO in each FP from fingers number 4, left hand in all the subjects. Our study analysed all fingers but we focused our attention upon fingers: 1, 4 and arbitrary 3.

We chose finger 1 for the following reasons:

- Modified Rodnan skin score is an international clinical score who has values between 0-51 points. For it’s quantification we do not calculate thickness of finger I skin, neither adjacent aria of finger I (for dorsum)
- When performing capillaroscopy, most authors (including us) excludes finger number I from both hands.

The arguments for choosing finger number 4 were capillaroscopy findings. Out of 25 consecutive SSc patients, 13 had the most advanced SSc characteristic capillaroscopy modifications in finger number 4; Fingers 3,5 and 2 had advanced SSc pattern in just 5, respective 4 and 3 patients.

We defined moment ONE as the second patients visit, after 2 months. For the moment one we included 10 patients.

We defined moment TWO as the third patients visit, after 6 months and we were able to gather 9 out of this 10 patients.
Determination of the environmental and intrinsic factors for the degradation in time of the biological material existing in fingerprints was carried out indirectly by studying the degradation stage and/or the epithelial cells disappearance. These were possible by examining the cytological samples, directly on fingerprint lifting material, colored with Nuclear Fast Red (NFR).

The estimative number of incorporated cells in each FP was determined by mathematical calculus, the values being obtained through the arithmetical rates of three observations, from random areas, where cells were counted. The constant element was the 20x objective surface of the Leica DMLS2 microscope which delimited the surface area where the cells were counted. [2-4].

The biological material, transferred on sterile cotton and washed, was concentrated by centrifugation. The DNA was extracted by using Chelex 5% solution, in the presence of Proteinase K, at 56 degrees Celsius. After lizing the epithelial cells to pH 11, the DNA macromolecules were purified by centrifugation and then by boiling in the presence of the ion changing resin. The DNA extract was put under the process of an advanced purification and concentration, by using Microcon YM.100 filters. The obtained deposit was suspended in 40 μl buffer solution Tris EDTA, pH 8,0 [5].

The DNA extract preparation with the view to be quantified was carried out by mixing each sample with 5 μl TaqMan Fast Univ PCR Master Mix, 1 μl Human DNA Quant Assay and 4μl of extract. The samples, being prepared that way, were put into the quantification system Fast Real Time PCR 7500 (Applied Biosystems, USA), including the positive and negative control and the standard calibration solutions. The data analysis was performed by using the Sequence Detection Software, version 1.3.1., 7500 Fast System SDS.

The result analysis and interpretation was carried out by using the device standard settings, including the software. The threshold was maintained at a constant value of 0.08730054, for all analyses, and the baseline between 3-15 cycles [6]. The extracted DNA amplification was carried out by using the Identifilter kit (Applied Biosystems, USA) with a PE 9700 thermocycler, at 34 cycles. The reaction mixture, for each sample, consisted in 5 μl PCR Reaction Mix, 2,5 μl Primer set, 0,25 μl AmplitaqGold DNA polymerase and 4,75 DNA extract.

**Results**

Microscopic studies showed that pores density in SSc patients had a significantly lower density when compared to controls in all 3 moments of our study (0, 1, 2); [Figure 6]

During the 30 days of follow up the FP in SSc patients and controls (moment ZERO), the pores start modifying their shape, and because of the papillary combs degradation, some pores disappeared; Pores density in SSc FP decreased faster in SSc FP than in controls FP.

*Papillary combs and grooves thickness* were measured by using Lucia Forensic application.

The papillary fingerprints kept in conditions of an **indoor environment**:

*After one day:*

Variation of *comb thickness in controls* was lower than in SSc patients.

The papillary fingerprints kept in conditions of an **outdoor environment:**

Variation of *comb thickness in controls* was lower than in SSc patients.

*The width of initial valleys (grooves thickness)* was lower in SSc FP than in controls.

At moments ONE and TWO of our study we noticed a worsening evolution process of SSc FP regardind papillary combs and grooves in 80% of the cases [Figure 2].
Desquamated epithelial cells

Showed regulate shapes and homogenous disposal in controls (Fig. 3).

Showed irregulate shapes and tendency of disposal in groups in SSc patients FP (Fig. 4). At moment ONE and TWO desquamated epithelial cells kept the tendency of disposal in groups in SSc patients versus homogenous disposal in controls.

DNA levels at moment ZERO

DNA levels in FP biological material were statistically significant higher in SSc patients vs. controls (mean = 210±67 vs. 118±49 pg per microl, p = 0,0002).

After one month of weekly following up the FP taken on glass, DNA levels decreased in SSc FP (from 210 to 103 pg per microl ) vs. controls (from 118 to 80 pg per microl) indicating a significant faster DNA degradation in SSc patients (p<0,0001).[Fig. 5]

DNA levels at moment ONE

FP obtained after 2 months showed higher DNA levels (mean¹=145±30 vs mean²=137±33) vs. DNA obtained at moment ZERO from SSc FP belonging to finger 4 left hand, but not significant (p=0,56591).

DNA levels at moment TWO

FP obtained after 6 months showed higher DNA levels (mean¹=140±30 vs mean²=137±33) vs. DNA obtained...
at moment ZERO from SSc FP belonging to finger 4 left hand, but also not significant.

Regarding all FP components described above we noticed:

*At moment ONE*: 8 out of 10 patients re-imprinted after 2 months showed in evolution degradation of morphological and biological constituents.

*At moment TWO*: 7 out of 9 patients re-imprinted after 6 months showed in evolution degradation of morphological and biological constituents.

**Structural modifications of the papillary relief in SSc**

The constituents of a SSc finger relief were: digital ulcers, digital scars and density of pores (Fig. 6).

There were no statistically significant associations between SSc FP constituents examined: pores density, thickness of papillary combs and grooves, epithelial cells disposal or DNA levels in any of the moment of our study (0, 1, 2) and clinical SSc features: age of patients, Rodnan score, onset of Raynaud, disease duration, SSc subsets, elevated phase reactants, active disease, synovitis, joint contractures, forced vital capacity, pulmonary hipertension, DLCO, scleredema, calcinosis, number of digital scars, capillaroscopic patterns.

In figure 7 we exposed both sides of SSc distal phalanga from two patients.

**Conclusions**

1. In all SSc fingerprints, pore density was lower, papillary combs were more oblatted and papillary grooves were narrower than in controls.
2. In SSc FP desquamated epithelial cells showed irregulate shapes and the tendency of disposal in groups versus regulate shapes and homogenous disposal in controls FP.
3. DNA levels were statistically significant higher but their decrease over time was faster in SSc FP versus controls FP. This can reflect that epithelial cells desquamation process is accelerated in SSc versus controls and also that SSc DNA from FP is worse qualitative versus controls DNA from FP.
4. On the second and the third visits, morphological constituents of FP were deteriorated versus first visit suggesting accelerated skin degradation in evolution of SSc.
5. Fingerprint evaluation is a non-invasive, simple, observational method and it may become the fundation of future attempt to find a fingerprint pattern capable to sustain the diagnosis of SSc in subjects with early scleroderma features.

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