

Collagen Specific Amino Acids in Skin in Localized Scleroderma

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Hydroxyproline, hydroxylysine and proline were determined on skin from 18 patients with localized scleroderma (10 with localized morphoea plaque and 8 with generalized morphoea). Three skin biopsies (4mm punch) were obtained from each patient: One from the center of a sclerotic plaque, one from the perilesional area, and one (control) from unaffected skin of the same region. Clinically, the sclerosis was more pronounced ($p < 0.01$) in localized morphoea plaque as compared to generalized morphoea. Patients with localized morphoea plaque had an increased concentration of hydroxylysine ($p < 0.01$) and an increased ratio of hydroxylysine to hydroxyproline ($p < 0.01$) in the plaques. Hydroxylysine concentration was not changed in patients with generalized morphoea. In the entire material, increased hydroxylysine concentration were related to shorter age of the plaques ($p < 0.05$) and to advanced degree of sclerosis ($p < 0.05$). The hydroxylysine and hydroxyproline content per mm² skin surface, and the weight of the dried defatted biopsy cores were increased in sclerotic plaques ($p < 0.01$) in localized as well as generalized morphoea. There were no changes in the hydroxyproline and proline concentrations in any of the groups. Specimens from perilesional area showed intermediate changes. The results were compared with selected cases of lichen sclerosis et atrophicus and atrophic skin diseases. The increase in hydroxylysine concentration and ratio to hydroxyproline indicate that patients with localized morphoea plaque contain an increased proportion of newly synthesized collagen in the fibrotic plaque. (Received April 5, 1983.)

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The amino acid hydroxylysine only appears in man in collagen. Hydroxyproline appears almost exclusively in collagen, but it is also found in elastic fibers. Proline is a normal constituent of a variety of proteins.

In sclerodermatous skin from patients with systemic sclerosis most authors find normal concentrations of hydroxyproline, although increased as well as decreased concentrations have been described (1, 2, 3, 4, 5, 6, 7). Cases with localized scleroderma have occasionally been included in previous studies of patients with the systemic form of the disease under the a priori assumption that the biochemical changes are identical in the two forms. Shuster et al. in one study reported increased hydroxyproline content in skin in a group of patients with morphoea, if related to skin surface area (8, 9). In 1979 Rodnan found increased weight of punch biopsy cores in scleroderma skin, and this easy method was recommended for quantification of the skin changes in scleroderma (10). Changes in hydroxylysine, the more collagen-specific amino acid, have only been studied in patients with systemic sclerosis (1, 4, 5, 6). Increased as well as decreased concentrations were reported.

Localized scleroderma is especially suited as a model in the study of sclerodermatous skin changes because specimens from scleroderma plaques as well as regional control of normal appearing skin can be obtained in the same individual minimizing regional differences and avoiding inter-individual differences.

In the present study, the relative and absolute content of hydroxyproline as well as hydroxylysine in skin is determined together with the content of proline in a group of patients with localized scleroderma, and in selected patients with lichen sclerosus et atrophicus and atrophic skin diseases for comparison.

MATERIALS AND METHODS

The material consists of 18 patients with a clinical and a histological diagnosis of localized scleroderma, i.e. 10 patients (8 women, 2 men) with a localized morphoea plaque, and 8 patients (7 women, 1 man) with generalized morphoea. The mean age was 28 years (range 17–59) among the patients with a localized morphoea plaque, and 57 years (range 36–75) among the patients with generalized morphoea.

Three skin biopsies (4 mm manual punch) were obtained under local anaesthesia with ethyl chloride freezing or subcutaneous injection of 1% lidocaine from each patient from

1. the center of a sclerotic plaque,
2. the perilesional area immediately outside the plaque,
3. normal appearing skin of the same region of the same individual.

The punch biopsies including epidermis/dermis were not trimmed.

Only plaques with the classical signs of scleroderma, i.e. circumscribed elements of white colour and increased dermal thickness and stiffness by palpation were studied. The plaques were located to the trunk in 10 cases, to the lower extremities in 5 cases, and to the arms in 3 cases. The regional control was obtained from the opposite side in 11 cases, and from the same side in 7 cases.

The mean age of the lesions was 3 ½ years (range ½–10) in patients with localized morphoea plaque, and 3 years (range ½–6) in patients with generalized morphoea. In the localized group, 6 lesions had progressed clinically during a 3 month-period before biopsy, and 5 exhibited a lilac ring. In the generalized form, 4 had progressed clinically, and 3 exhibited a lilac ring. Four lesions of each group showed pigmentation. Clinically, 6 lesions in the localized group and 4 in the generalized group were concluded to be progressive and active, while 4 lesions in each group were concluded to be stationary. As shown in Table 1, the degree of sclerosis on clinical examination prior to biopsy was significantly ($p < 0.01$) more pronounced in patients with localized morphoea plaque as compared to patients with generalized morphoea.

Biopsies after the principles described above were also performed in 3 patients with lichen sclerosus et atrophicus (2 sets of biopsies in one of the patients), and in 3 patients with atrophic skin diseases, i.e. 2 patients with local panatrophy with pigmentation of the skin and underlying atrophy of the subcutaneous tissue, the muscle and the bones (2 sets of biopsies in one of the patients), and 1 patient with atrophic morphoea with pigmentation and primary atrophy of the skin and slight atrophy of the underlying subcutaneous tissue.

Collagen analyses

The skin samples were extracted with acetone and ether and dried under vacuum. The weight of the dried, defatted tissue was determined on a Mettler® scale. The dried and defatted skin was hydrolyzed in 6 N HCl for 18 hours at 118°C. The hydrolysate was evaporated to dryness at 60°C at 50 mbar. The content of hydroxyproline, hydroxylysine and proline of the hydrolysate was measured in a Technicon AutoAnalyzer® apparatus ad modum Blumenkrantz & Asboe-Hansen and related to defatted dried weight of the biopsy core (11, 12).

Table 1. Degree of sclerosis of morphoea plaques at clinical examination, and duration of the plaques at biopsy

Degree of sclerosis	Number of plaques					
	Localized morphoea (n=10)			Generalized morphoea (n=8)		
	<2 years	≥2 years	Total	<2 years	≥2 years	total
Slight	0	0	0	1	5	6
Advanced	6	4	10	2	0	2

LOCALIZED MORPHOEA PLAQUE

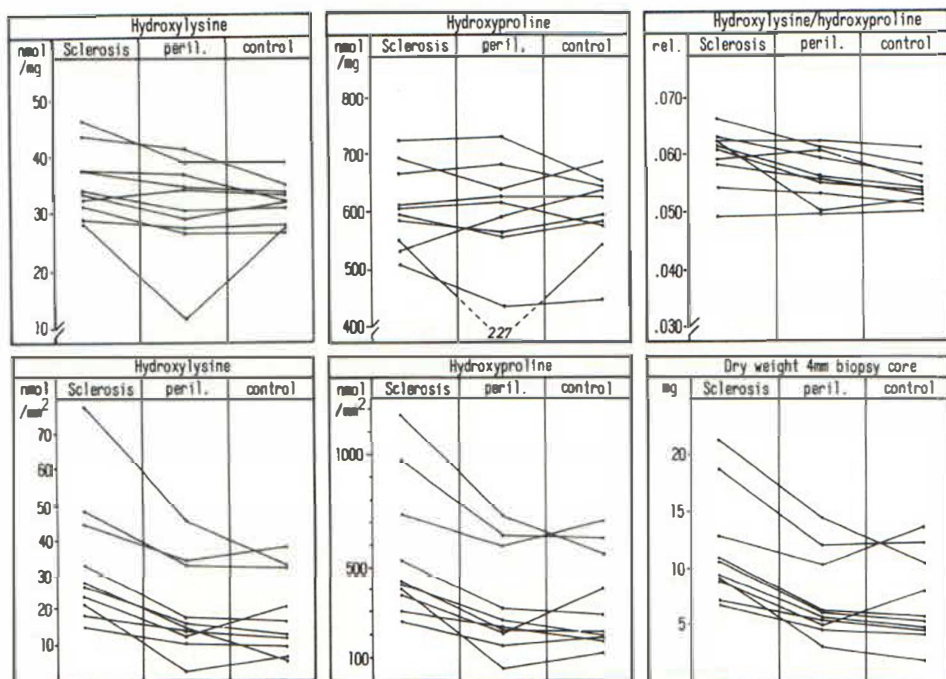


Fig. 1. Localized morphoea plaque: Collagen specific amino acids in sclerotic area, perilesional area and regional control expressed as concentration (nmol per mg defatted, dried skin), and related to skin surface area. The dried, defatted weight of 4 mm punch biopsies, and the molar hydroxylysine/hydroxyproline ratio are also shown. $N=10$.

The accuracy of the collagen analysis was controlled daily with a collagen-standard of guinea pig skin. Analyses of hydroxyproline, hydroxylysine, and proline had a reproducibility of 1%, 2%, and 1%, respectively.

Statistical methods

The Student's *t*-test was used on the paired differences between values from sclerotic area and the regional controls (13). Fisher's exact test for 2 by 2 tables was used for analyses of the relation between hydroxylysine concentration and age of the plaques.

RESULTS

The results of determinations of hydroxyproline (Hyp), hydroxylysine (Hyl) and proline (Pro) concentrations (nmol/mg dried, defatted skin) and the molar ratio between Hyl and Hyp in localized morphoea plaque and in generalized morphoea are shown in Figs. 1–2. The figures also include determinations of dried, defatted weight of the 4 mm punch biopsy cores (DW), and Hyp and Hyl related to skin surface area (nmol/mm²) according to the determination of DW.

The Hyp and the Pro concentrations were not different in the sclerotic area as compared to the regional control. The Hyl concentration and the Hyl/Hyp ratio were increased in localized morphoea plaque ($p<0.01$), but not in generalized morphoea as compared to regional control. The numerical differences, however, were small. In the entire material increased Hyl concentration was related to shortest age of the plaques ($p<0.05$), and to

GENERALIZED MORPHOEA

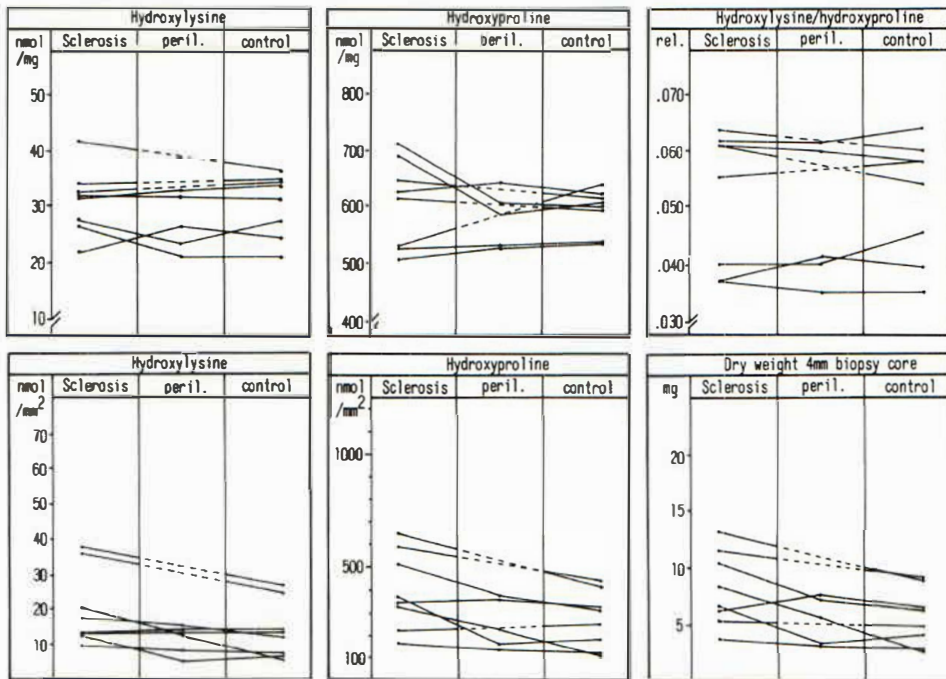


Fig. 2. Generalized morphoea: Collagen specific amino acids in sclerotic area, perilesional area and regional control expressed as concentrations (nmol per mg defatted, dried skin), and related to skin surface area. The dried, defatted weight of 4 mm punch biopsies, and the molar hydroxylysine/hydroxyproline ratio are also shown. $N=8$.

advanced degree of sclerosis ($p<0.05$) (Table II). The Hyl concentration was not related to clinical signs of progression or lilac ring, and there was no statistical relation between age of the plaques and degree of sclerosis.

DW and Hyp and Hyl related to skin surface area were increased in localized morphoea plaque ($p<0.01$) as well as generalized morphoea ($p<0.05$) as compared to normal appearing skin of the same region. In the entire material of patients with morphoea, an increase in Hyl related to skin surface area was the more pronounced ($p<0.001$).

Table II. Difference in hydroxylysine concentration (expressed in nmol per mg defatted, dried skin) between sclerotic plaques and regional control related to duration of localized scleroderma, and to degree of sclerosis clinically

	Hydroxylysine concentration, nmol/mg		
	Median	Range	
Duration			
<2 years	3.6	-2.1-8.5	($n=9$)
≥ 2 years	0.8	-2.4-5.5	($n=9$)
Degree of sclerosis			
Slight	-0.2	-2.4-5.1	($n=6$)
Advanced	1.9	-2.1-8.5	($n=12$)

LICHEN SCLEROSUS ET ATROPHICUS

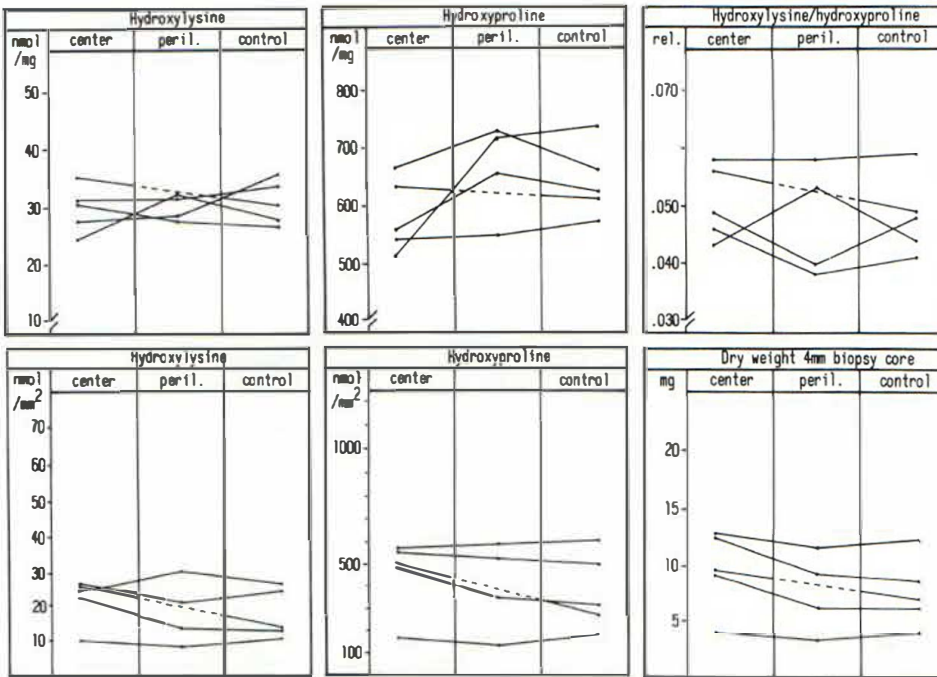


Fig. 3. Lichen sclerosis et atrophicus: Collagen specific amino acids in sclerotic area, perilesional area and regional control expressed as concentration, (nmol per mg defatted, dried skin), and related to skin surface area. The dried, defatted weight of 4 mm punch biopsies, and the molar hydroxylysine/hydroxyproline ratio are also shown.

Analyses of the perilesional area showed intermediate values.

A summary of the results in morphea patients is presented in Table III.

The results of collagen analyses in patients with lichen sclerosis et atrophicus are presented in Fig. 3. There were no differences in any of the parameters studied among the areas.

The results of collagen analyses in patients with atrophic conditions are presented in

Table III. Collagen specific amino acids, proline and DW in morphea

Statistical analysis of values from sclerotic area compared with regional control

Localized scleroderma sclerotic area/control	Loc. morphea plaque n=10	Generalized morphea n=8	Total n=18
Hyp, nmol/mg	NS	NS	NS
Hyl, nmol/mg	**	NS	**
Pro, nmol/mg	NS	NS	NS
Hyl/Hyp, rel.	**	NS	**
DW, mg	**	*	**
Hyp, nmol/mm ²	**	*	**
Hyl, nmol/mm ²	**	*	***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

ATROPHIC CONDITIONS

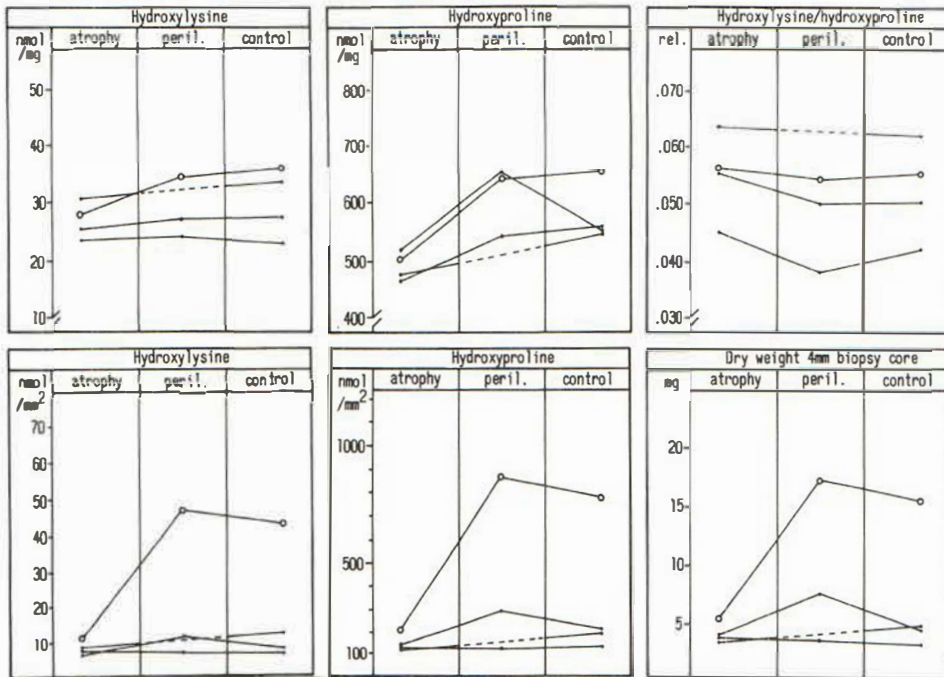


Fig. 4. Local panatropy (●) and atrophic morphoea (○): Collagen specific amino acids in sclerotic area, perilesional area and regional control expressed as concentrations (nmol per mg defatted, dried skin), and related to skin surface area. The dried, defatted weight of 4 mm punch biopsies, and the molar hydroxylysine/hydroxyproline ratio are also shown.

Fig. 4. In the case of atrophic morphoea the Hyp and Hyl concentration, DW, Hyp and Hyl related to skin surface area were much lower in the atrophic area, while the Hyl/Hyp ratio seemed unchanged. The patients with panatropy showed only minimal changes in the direction of lower values in the pigmented areas.

DISCUSSION

The increases in the Hyl concentration and the molar ratio to Hyp in patients with localized morphoea plaque may indicate an increased proportion of newly synthesized collagen in the fibrotic plaques, as studies in chicks have shown a relative and temporary increase in Hyl during the embryogenic period (14, 15). Increased Hyl concentration was also found in dermal scar tissue and in healing bone fractures (16, 17). Hydroxylysine molecules participate in collagen cross-linking after aldehyde formation, and a significant number of hydroxylysine molecules have, probably, not yet participated in this linking in scleroderma skin. The increase in Hyl concentration was related to shorter duration of the morphoea plaques and to advanced degree of sclerosis. The Hyl concentration and the ratio to Hyp were not increased in generalized morphoea. The age of the elements and the clinical signs of progression were comparable in the two forms, but the degree of sclerosis was significantly less pronounced in generalized morphoea.

DW and the surface-related measurements of Hyp and Hyl were increased in localized

morphoea plaque as well as generalized morphoea, and they were, thus, compared with Hyl concentration, more sensitive as expressions of fibrosis in morphoea. As a consequence of the increased DW and Hyl concentration, Hyl related to skin surface area was the more sensitive expression of fibrosis among the parameters studied. DW is easy to measure and suited for quantification of scleroderma as previously reported by Rodnan, but Hyl related to skin surface area is more sensitive and more specific (10).

In this study of localized scleroderma specimens from fibrotic plaques, perilesional areas, and regional control were taken from the same individual minimizing regional variations and avoiding inter-individual, age- and sex differences. Considerable differences in Hyl, Hyp, and Pro concentrations were found between skin from the wrist and the groin, and large age- and inter-individual differences were found too (18). Inter-individual, age, and regional differences may explain why the increase in Hyl concentration was not found previously in studies of the systemic form of scleroderma.

The increase in DW indicates that the skin thickness is increased in sclerotic plaques. Punch biopsy is an invasive method, which cannot be repeated at the same site; it cannot be used in the fingers of patients with acrosclerosis. Precise non-invasive methods applicable to a variety of anatomical regions for measurements of skin and soft tissue thickness are of interest as for quantification of the skin changes in scleroderma.

We found no changes in collagen-specific amino acids in patients with lichen sclerosus et atrophicus, where the fibrotic process is located only to a narrow band in the outer dermis. In a punch biopsy representing the full thickness of the dermis, the fibrotic band in lichen sclerosus et atrophicus will constitute a minor fraction only.

The patient with atrophic morphoea exhibited decreased collagen-specific amino acids and DW, the reverse situation of morphoea in the sclerotic state. In local panatropy no evident changes were found in the analyses of skin, and the condition was dominated by the atrophy of subcutaneous tissue and muscle.

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