Dehydroepiandrosterone sulphate serum levels in systemic sclerosis

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Abstract

Objective

To evaluate in a cohort of women with systemic sclerosis (SSc) the dehydroepiandrosterone sulphate (DHEAS) serum levels and their relationship with disease severity.

Methods

DHEAS serum concentrations were measured by radioimmunoassay in 40 SSc patients and compared with those in 40 controls matched for sex and reproductive status. IL-2 sR was evaluated as a disease activity index. A preliminary organ/system severity scale proposed by Medsger et al. *in 1999 was used to evaluate disease severity.*

Results

Mean serum levels of DHEAS in SSc women of childbearing age were significantly lower than in controls (0.87 ± 0.85 g/ml versus 2.75 ± 0.42 g/ml; p < 0.001). On the contrary, no difference was found between postmenopausal women and controls. A reduction below the 95% confidence limits was found in 10 out of 11 patients of childbearing age and in 8 out of 29 postmenopausal women, respectively. In 5 out of 11 patients of childbearing age taking steroids for their SSc (< 10 mg/daily) DHEAS levels were significantly lower than in patients not taking steroids (p = 0.01). On the contrary, 16 out of 29 postmenopausal women using steroids had lower DHEAS concentrations than in patients not taking steroids, although the difference was not statistically significant. There was no statistically significant difference in DHEAS levels between patients with diffuse or limited SSc, or between those with or without organ system involvement. No correlations were found either in pre- and post-menopausal steroid nonusers, or in limited and diffuse subsets, between DHEAS levels and age, postmenopausal years, disease duration, IL-2 sR , disease organ/system severity scale.

Conclusion

Our data show that, as in other autoimmune diseases, low serum DHEAS is a feature of premenopausal SSc patients. More extensive prospective studies are needed to define the exact role of DHEAS dysregulation in SSc.

Key words

Dehydroepiandrosterone sulphate, systemic sclerosis.

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Introduction

Systemic sclerosis is a multi-system connective tissue disease characterised by skin sclerosis and fibrosis of the internal organs. A clear picture of the etiopathogenesis of the disease is lacking, but genetic and immunologic mechanisms seem to play an important role (1).

Recently, it has been proposed that adrenocortical hormone dehydroepiandrosterone sulphate (DHEAS) may be implicated in the pathogenesis of some immunological diseases, including systemic sclerosis (SSc). In fact, DHEAS levels have been found to be reduced in the serum of patients with active SSc (2), as well as in polymyalgia rheumatica/giant cell arteritis, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), ulcerative colitis, Crohn's disease, human immune deficiency virus (HIV) infection (3-10), and in some dermatological disorders. On the contrary, normal values have been reported in ankylosing spondylitis, fibromyalgia, osteoarthritis, and psoriatic arthritis (3-5). It has been suggested that DHEAS may have an immunoregulatory effect, and thus its reduction could play a role in the pathogenesis of the autoimmune diseases.

In this study we evaluated DHEAS levels in SSc patients compared with healthy subjects, and their relationship with disease severity.

Table I. Epidemiological and clinical features of SSc patients.

Systemic sclerosis	40 females
Age (years ± SD)	49.5 ± 13.4
Disease duration (years ± SD)	13.1 ± 9.5
Childbearing age women	11 cases
Postmenopausal women	29 cases
Clinical subsets of SSc*	
Sine scleroderma	2 cases
Limited	11 cases
Intermediate	18 cases
Diffuse	9 cases
Clinical subsets of SSc	
Limited	31 cases
Diffuse	9 cases
Autoantibodies	
Anticentromere	6 cases
Anti Scl-70	29 cases
ANA others	5 cases

*according to (12); **according to (13).

Materials and methods

Patients

We evaluated 40 female SSc patients attending our Rheumatology Unit, who fulfilled the preliminary criteria for the classification of systemic sclerosis (11). Table I summarizes the clinical and demographic data on the study group. Their mean age was 49.5 ± 13.4 years (range 15-72 years) and their disease duration was 13.1 ± 9.5 years (range 1-34 years), considering the appearance of Raynaud's phenomenon or another characteristic symptom of SSc as the point of disease onset. A total of 29/40 SSc patients were postmenopausal, with menopause occurring at the age of 46.2 ± 6 years (range 30-56 years); the number of years post-menopause in these patients ranged from 7

Table. Ia. Organ system involvement in 40 SSc patients.

Organ/system involved*	No. of cases	Limited (n = 31)	Diffuse (n = 9)
General	21	15 (48.3%)	6 (66.7%)
Peripheral vascular	35	26 (83.8%)	9 (100%)
Skin	38	29 (93.5%)	9 (100%)
Joint/tendon	11	9 (29.0%)	2 (22.2%)
Muscle	0	0	0%
GI tract	39	30 (96.8%)	9 (100%)
Lung	39	30 (96.8%)	9 (100%)
Heart	20	16 (51.6%)	4 (44.4%)
Kidney	4	2 (6.4%)	2 (22.2%)

*According to Medsger *et al.* (14).

months to 30 years (mean 9.7 ± 7.2 years).

Furthermore, the patients were classified in 4 subsets according to the criteria of Giordano *et al.* (12), which are based on the extent of skin involvement; there were 2 cases of SSc *sine* scleroderma (ss), 11 cases of limited (lc) SSc, 18 cases of intermediate (i) SSc, and 9 cases of diffuse (dc) SSc. According to the subset criteria of LeRoy *et al.* (13) our patients could be grouped in 31 cases with limited SSc and 9 cases with diffuse SSc.

In the analysis of antinuclear autoantibodies (ANA), 6 patients showed an ACA pattern detected by the immunofluorescence technique using the Hep2 cell line as substrate; 29 patients showed anti-topoisomerase I (anti Scl-70) antibodies by ELISA; and ANA were negative for ACA and anti Scl-70 antibodies in 5 cases.

The patients were receiving prednisone (<10 mg/day) in 20 cases, non-steroidal anti-inflammatory drugs in 12 cases, D-penicillamine (300 - 600 mg/day) in 5 cases, griseofulvine (1000 mg/day) in 5 cases, hydroxychloroquine (400 mg/day) in 2 cases, pentoxifylline (800 - 1200 mg/day) in 19 cases, ACE-inhibitors (20 mg/day) in 2 cases, cisapride (40 mg/day) in 26 cases, ranitidine (150 mg/day) in 6 cases, and omeprazole (20 mg/day) in 17 cases.

The control group consisted of 40 healthy subjects matched for sex and reproductive status (pre and postmenopausal). Informed consent was obtained from all patients and controls in accordance with the guidelines of the Declaration of Helsinki for medical research.

Methods

Blood samples were collected from each SSc patient and control subject in the morning between 8:00 and 9:00 at rest (basal conditions). After centrifugation, the serum specimens were stored at -30°C until assayed. DHEAS was measured by a standardised radioimmunoassay (RIA) method according to the manufacturer's instructions for the DHEAS serum level determination (Radim Rome, Italy). The hormonal limit of detection was 0.02 g/ ml.

As the disease activity index, soluble interleukin 2 receptor (IL-2 sR) was measured using an immunometric enzyme immunoassay (ELISA) (Quantikine mRNA, R & S Systems Europe Ltd.). The limit of detection was less than 6 pg/ml.

At the same time a clinical evaluation was carried out. Disease severity was assessed using the preliminary nine organ/system (general, peripheral vascular, skin, joint/tendon, muscle, gastrointestinal (GI) tract, lung, heart, and kidney) severity scale (range 0-4) for SSc proposed by Medsger *et al*. (14).

To detect internal organ involvement, the following examinations were performed: electrocardiogram, doppler echocardiography, M/B mode echocardiography, capillaroscopy, pulmonary function tests, high resolution computed tomography of the chest, esophageal and/or gastrointestinal barum study, and a xylose absorption test. Skin involvement was evaluated by measuring the total skin thickness according to Rodnan *et al*. (15).

Table II shows the prevalence of cases grouped according to the disease severity scale for systemic sclerosis.

None of the patients were smokers. No patient was on treatment with drugs that could reduce DHEAS serum concentrations, such as estrogen oral contraceptives (16); none had taken drugs that could increase DHEAS serum levels, such as calcium channel blockers (17, 18), during the week preceding serum collection for the hormonal assay. Clinically, the patients were not affected by psychiatric or mental disorders (19, 20).

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) (version 6.1 for Windows). Values are expressed as the mean ± standard deviation (SD). Fisher's exact test was used for the frequency analysis, and the non-parametric Wilcoxon rank sum test to compare the mean DHEAS levels among SSc subgroups and with respect to the controls. A p value < 0.05 was considered significant. Multiple linear regression analysis was performed to assess the effect of DHEAS on IL-2 sR , and on the scleroderma organ system severity scale in pre- and post-menopausal patients not taking steroids, and in patients with limited and diffuse SSc. Spearman's correlation coefficient analysis was used to test significance according to Bonferroni's correction (alfa = 0.05/number of comparisons).

Table II. Organ system severity scale in 40 SSc women*.

Organ system involvement	0 (Normal)	1 (Mild)	2 (Moderate)	3 (Severe)	4 (End stage)
General	19 (47.5%)	17 (42.5%)	4 (10%)	0 (0%)	0 (0%)
Peripheral vascular	5 (12.5%)	8 (20%)	12 (30%)	15 (37.5%)	0 (0%)
Skin	2 (5%)	21 (52.5%)	14 (35%)	2 (5%)	1 (2.5%)
Joint/tendon	29 (72.5%)	2 (5%)	6 (15%)	1 (2.5%)	2 (5%)
Muscle	40 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
GI tract	1 (2.5%)	34 (85%)	5 (12.5%)	0 (0%)	0 (0%)
Lung	1(2.5%)	11(27.5%)	14 (35%)	14 (35%)	0 (0%)
Heart	20 (50%)	3 (7.5%)	15 (37.5%)	0 (0%)	2 (5%)
Kidney	36 (90%)	1 (2.5%)	3 (7.5%)	0 (0%)	0 (0%)

*According to Medsger *et al*. (14).

Results

The mean serum levels of DHEAS are shown in Table III. In SSc patients of childbearing age, DHEAS levels were significantly lower than in controls, i.e. 0.87 ± 0.85 g/ml versus 2.75 ± 0.42 g/ml) ($p < 0.001$). In postmenopausal women their mean concentrations (0.45 ± 0.40 g/ml) were not statistically different from the controls (0.50 ± 0.25 g/ml). Blood hormonal levels were found to be lower than in controls in 45% of SSc women. DHEAS concentrations below the 95% confidence limits were found in 10 out of 11 patients of childbearing age (90.9%) and in 8 out of 29 postmenopausal women (27.5%), respectively (Fig. 1). Other patients had DHEAS concentrations within the normal range.

Table III. DHEAS serum levels in SSc patients of childbearing age, postmenopausal SSc patients, and controls.

Women	No. of cases	DHEAS (g/ml) SSc	Controls	P*
Childbearing age	11	0.87 ± 0.85	2.75 ± 0.42	< 0.001
Postmenopausal	29	0.45 ± 0.40	0.50 ± 0.25	ns

*Wilcoxon sum rank test.

Table IV. DHEAS serum levels in SSc patients on steroids and those not on steroids.

SSc women	No. of cases	DHEAS (g/ml) Steroid users	Steroid non-users	P*
Childbearing age	11	0.24 ± 0.09 (n=5)	1.4 ± 0.83 (n=6)	0.01
Postmenopausal	29	0.39 ± 0.4 (n=16)	0.54 ± 0.36 (n=13)	ns

*Wilcoxon sum rank test; the number of cases is shown between parentheses.

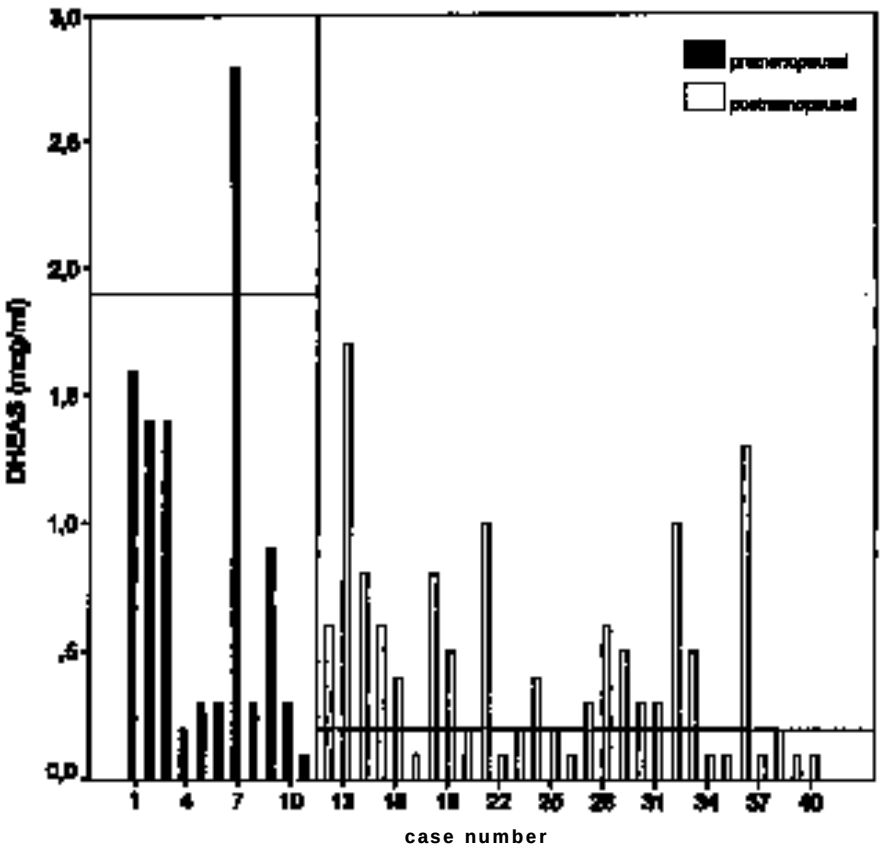

Fig. 1. Distribution of DHEAS serum levels in 11 SSc patients of childbearing age and in 29 postmenopausal SSc patients. Horizontal lines indicate the lower confidence limits for the two groups.

In the analysis of the effects of corticosteroid use, in 5 out of 11 childbearing SSc women (45.4%) taking steroids DHEAS levels were significantly lower than in similar patients not taking steroids (0.24 ± 0.09 g/ml versus 1.4 ± 0.83 g/ml) ($p = 0.01$) (Table IV). On the contrary, in 16 out of 29 postmenopausal patients using corticosteroids (55.2%), DHEAS levels were not significantly lower than in nonuser patients (0.39 ± 0.4 g/ml versus 0.54 ± 0.36 g/ml, respectively) ($p > 0.05$) (Table IV).

IL-2 sR serum levels were 1508 ± 270 pg/ml (range 146.8 pg/ml to 9608 pg/ml). Seven out of 40 SSc patients (17.5%) showed levels greater than the 95% confidence limits.

The SSc patients, divided into the subsets of limited and diffuse SSc according to the criteria of Le Roy *et al.*, were not significantly different when age (50.9 ± 12.4 years versus 44.8 ± 16.1 years, respectively; $p > 0.05$), duration of menopause (10.2 ± 7.3 years versus 7.7 ± 7.1 years, respectively; $p > 0.05$), or the frequency of organ system involvement were considered (Table Ia). On the contrary, a statistically significant difference was found with respect to the disease duration, which was longer in limited than in diffuse SSc (15 ± 9.6 years versus 6.8 ± 5.4 years, respectively) ($p = 0.02$).

No significant differences were found when DHEAS concentrations in the limited and diffuse SSc subgroups were compared (0.58 ± 0.60 g/ml versus 0.52 ± 0.49 g/ml, respectively) ($p > 0.05$). Moreover, no statistically significant clinical difference was found between patients with low or normal DHEAS levels (Table V). Dividing the patients according their median skin score (< 12.5 versus ≥ 12.5) and according to the median organ/system involvement (< 4 versus ≥ 4, respectively), statistically significant differences of DHEAS levels (n = 20: 0.67 ± 0.1 g/ml versus n = 20: 0.47 ± 0.09

Table V. Organ system involvement in SSc-patients divided according to normal and low DHEAS serum levels.

Organ system involvement	DHEAS serum levels Normal (n = 22)	Low (n = 18)
General	50%	44.4%
Peripheral vascular	100%	100%
Skin score	77.3%	88.8%
Joint/tendon	18.2%	22.2%
GI tract	81.8%	94.4%
Muscle	0%	0%
Lung	81.8%	94.4%
Heart	40.9%	61.1%
Kidney	4.5%	5.5%

g/ml, $p > 0.05$; n = 15: 0.6 ± 0.2 g/ml versus n = 25: 0.5 ± 0.1 g/ml, $p > 0.05$, respectively) were pointed out.

In both the pre- and postmenopausal SSc groups not on corticosteroids, and in both the limited and diffuse SSc subsets, DHEAS levels were not significantly associated with age, postmenopausal years, disease duration, IL-2 sR serum levels, and scleroderma organ system severity scale (Spearman's rank correlation).

Multiple regression analysis to assess the relative role of DHEAS levels in SSc organ/system involvement, performed in patients not on corticosteroids according to reproductive status (pre- and postmenopausal), and disease subset (limited and diffuse) showed no significant associations.

Discussion

This study shows decreased DHEAS levels in premenopausal SSc patients, and confirms that corticosteroid treatment is an important factor to consider in patients with reduced DHEAS levels, because steroids cause suppression of pituitary ACTH secretion (21, 22). Our data did not show statistically different hormonal levels in patients divided according to SSc clinical subset, median skin thickness score, or median organ/system involvement. Nor were statistically significant correlations, both in pre- and postmenopausal patients not on corticosteroids, seen between DHEAS levels and variables such as disease duration, IL-2 sR , or the scleroderma organ system severity score recently proposed by Medsger *et al.* (14). This behaviour suggests that DHEAS dysregulation may be a feature of the disease, but that it does not play a role in tissue damage. Nevertheless, these results are in contrast with those of Straub *et al.* (2), who demonstrated a negative association between DHEAS concentrations and disease severity, detected according to the number of disease manifestations. This discrepancy may be linked to the different method used to quantify disease severity.

In mental disorders, such as neurasthenia and schizophrenia, DHEAS levels have been shown to be depressed (19, 20). Our patients were not affected by these disorders,nor did they show signs of depression, although no psychometric tests were performed.

On the whole, our data are in accordance with published studies showing low serum DHEAS concentrations in various autoimmune diseases, including SSc (2-9, 23). The low DHEAS levels seen in our premenopausal SSc patients, such already shown in premenopausal-onset RA (23-25) also might have a fundamental role in the pathogenesis of the diseases, as has been proposed for other connective tissue diseases (26, 27).

It has been suggested that DHEAS, in addition to its questionable effects on the cardiovascular system, coagulation, and metabolism as reviewed by Derksen (9), may regulate some immune reactions. Epidemiological data have shown that the decline in immunocompetence during aging correlates with decreasing serum DHEAS concentrations (28). DHEA specific receptors and DHEA stimulation of interleukin-2 (IL-2) production by T cells (29, 30) have been reported. DHEAS has been found to suppress the activity of the human IL-6 gene promoter (31, 32), thus suggesting protective anti-inflammatory/ immunosuppressive effects.

Several data indicate that androgen steroids play a prominent role in physiological and pathological conditions involving the vascular endothelial system (26). Therefore, as has been hypothesised for RA (32), low levels of DHEAS, generally downregulating the immune response, could play a role in the vascular dysfunction associated with inflammation which represents an important pathological feature of SSc (1).

The absence of any correlation in this study between DHEAS levels, disease severity and disease activity, when IL-2 sR was considered, might indicate that when chronically low hormonal levels occur they signify a fundamental risk factor for occurrence of the disease. Thus, hormonal dysregulation could result in relative immunological hyperreactivity with increased cytokine levels before disease onset, contributing to increased susceptibility to SSc in genetically predisposed patients.

With respect to these considerations, Raynaud's phenomenon (a typical early manifestation of SSc) and permanent capillary abnormalities (which often appear later) could be explained by the initial and chronic vascular instability induced by hormonal dysregulation. The downregulation of specific inflammatory cytokines reported to be present in abnormally high amounts in SSc patients (IL-1, IL-2, IL-4, IL-6, IL-8, tumor necrosis factor (TNF)- , transforming growth factor (TGF)- , platelet derived growth factor (PDGF), and leukotriene b4) (33) can alter the functions of vascular cells and fibroblasts, resulting in the accumulation of collagen and other extracellular matrix constituents (34).

In rheumatic patients, improvement in both clinical symptoms and laboratory parameters of disease were correlated with increases in DHEAS levels (35, 36), as well as an improvement in disease activity when a pharmacological dosage of dehydroepiandrosterone (DHEA) was introduced (37). Thus, because it has been suggested that DHEA administration to SLE and RA patients may exert positive effects (26, 36, 37), their use in SSc patients with low androgen serum levels could be considered as well. Nevertheless, because the immunological effects of DHEA in humans have not been studied adequately, other and more extensive prospective studies may be needed to define more clearly the role of

DHEAS dysregulation in SSc, in order to evaluate whether low hormonal values contribute only to the immune disorder and/or tissue damage.

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