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ORIGINAL ARTICLE

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Adrenocorticotropic hormone and dehydroepiandrosterone sulfate levels of rheumatoid arthritis patients treated with glucocorticoids

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Abstract To assess adrenal function with respect to the presence or absence of steroid therapy, we investigated differences in the blood levels of adrenocorticotropic hormone (ACTH) and dehydroepiandrosterone sulfate (DHEAS) in relation to steroid (prednisolone) administration in 123 patients with rheumatoid arthritis (RA). Levels of ACTH and DHEAS were significantly lower in the steroid-treated group than in the non-treated group (ACTH: 11.79 pg/ml vs 27.92 pg/ml) (DHEAS: 418.12 ng/ml vs 883.91 ng/ml) ($P < 0.0001$). We observed no steroid dose-related differences in ACTH levels. However, DHEAS levels showed a slight decrease at a prednisolone dose of 2.5 mg/day, with a significant decrease being observed at a dose of 5 mg/day when statistical adjustments were made for age and sex ($P < 0.0001$). At doses of 7.5 mg/day or greater, DHEAS levels were significantly lower than those for 5 mg/day ($P < 0.0006$). These results suggest that low-dose prednisolone reduces adrenal function in patients with RA. We recommend that doses of prednisolone should be limited to 5 mg/day or less in consideration of adrenal function when treating RA patients. The measurement of ACTH and DHEAS may be useful for evaluating adrenal function in patients with RA.

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Introduction

Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) are secreted by the adrenal cortex and are classified as sex hormones.[1] Dehydroepiandrosterone sulfate levels markedly increase during adolescence, and reach a peak at about 20 years of age, after which they decrease with age. As such, DHEAS may be used as a parameter of aging.[2,3] Previous studies have suggested that DHEAS prevents osteoporosis, cardiovascular disease and arteriosclerosis, and it is becoming increasingly recognized that DHEAS is important for maintaining a healthy state.[4,5] In patients with rheumatoid arthritis (RA), there have been reports of normal, increased, and decreased DHEAS values.[6–9] However, steroids are often administered to these patients, and may decrease the levels of adrenocorticotropic hormone (ACTH) and DHEAS via negative feedback mechanisms.[1] Furthermore, a previous study has reported that blood levels of DHEAS are constant for a long period,[8] but that mental or physical stress and inflammatory responses may influence DHEAS levels via ACTH.[1,10] In this study, we investigated differences in the blood levels of ACTH and DHEAS in subjects on and off of various doses of steroids. In addition, we examined changes in ACTH and DHEAS levels in 14 patients for whom these parameters were measured twice.

Patients and methods

Of 130 RA patients meeting the criteria established by the American College of Rheumatology,[11] for whom levels of ACTH and DHEAS were measured between February 2002 and September 2002, there were 123 patients (137

samples) for whom confirmation of the undertaking and dosages or non-undertaking of prednisolone therapy could be made (patients treated with betamethasone were excluded). In the 14 patients for whom these parameters were measured twice, the values at the second measurement were also evaluated. Subjects were 123 patients (26 males, 97 females) consisting of 19 inpatients and 104 outpatients, with a mean age of 57.24 years (±12.58) at the time of examination. Steroids had been administered to 78 of these patients, the daily prednisolone doses were 2.5 mg in 16 patients, 5 mg in 47 patients, 7.5 mg in 9 patients, and 10 mg in 6 patients (Table 1).

Adrenocorticotropic hormone levels were determined by a one-step IRMA (immunoradiometric assay) method using biotinylated antibodies, and, for B/F (bound/free) isolation, avidin-bound beads were used (Allegro HS-ACTH IRMA kit: Nichols Diagnostics, San Juan Capistrano, CA, USA). Serum DHEAS was determined after serum was allowed to react competitively with I-labeled DHEAS in a DHEAS antibody coated tube (DHEA-S kit: Coat-A Count DHEA-SO_4, Mitsubishi Kagaku Iatron, Tokyo, Japan). In addition, we investigated the correlations of ACTH and DHEAS values with age, erythrocyte sedimentation rate (ESR), matrix metalloproteinase (MMP_3), red blood cell count (RBC), and rheumatoid factor (RF). Blood samples were measured between 08:30 and 11:00 h. Before the measurement of these values, informed consent was obtained from all patients.

Reproducibility of ACTH and DHEAS measurements

Using 3 samples of ACTH with different concentrations, Intra-day ACTH concentrations were evaluated 10 times on 1 day. The coefficients of variation (CV) were 3.8%, 2.4%, and 2.2%. Inter-day ACTH concentrations were evaluated daily over a period of 5 days. The CV were 1.3%, 5.1%, and 3.6%. Using 3 samples of DHEAS with different concentrations, intra-day DHEAS concentrations were evaluated 20 times on 1 day. The CV were 7.2%, 4.7%, and 3.4%. Inter-day DHEAS concentrations were evaluated daily over a period of 10 days. The CV were 10.6%, 4.9%, and 4.2%. These values confirmed the reproducibility of ACTH and DHEAS measurements.

Table 1. Characteristics of 123 rheumatoid arthritis (RA) patients treated with various doses of prednisolone

Steroid dose (mg)	No. of cases	Sex		Age (years)[a]
		Male	Female	
0	45	6	39	54.76 ± 12.37
2.5	26	1	15	61.13 ± 7.91
5	47	11	36	56.45 ± 14.65
7.5	9	5	4	60.33 ± 8.02
10	6	3	3	67.00 ± 3.03
Total	123	26	97	57.24 ± 12.58

[a] Mean ± SD

Statistical analysis

Differences between the ACTH and DHEAS levels of steroid-treated and non-treated RA patients were compared using a Wilcoxon rank sum test. In addition, the effects of steroids on ACTH and DHEAS levels were evaluated by analysis of variance with steroid dosage as a factor, and by analysis of covariance with steroid dosage as a factor and with age and sex as covariates. Prior to the comparison, it was confirmed that there were no correlations between ACTH or DHEAS levels and age, CRP, ESR, MMP_3, RBC, or RF through simple correlation coefficients and partial correlation coefficients. P values of less than 0.05 were considered to be significant.

Results

Differences in ACTH and DHEAS levels related to the steroid administration and dosage

In the steroid-treated group, ACTH and DHEAS levels were lower (Wilcoxon's test, $P < 0.0001$) (Table 2) than the non-treated group. There were no dose-related differences in ACTH levels (Table 3). However, DHEAS levels in the steroid-treated group were lower than in the non-treated group at a prednisolone dose of 2.5 mg/day (Table 4), and significant decreases in DHEAS levels were observed at doses of 5 mg/day ($P < 0.0002$) or greater after adjustments were made for age and sex (Table 4). In addition, at doses of 7.5 mg/day or higher, DHEAS levels were significantly lower than at doses of 5 mg/day ($P < 0.0006$).

Table 2. Adrenocorticotropic hormone (ACTH) and dehydroepiandrosterone sulfate (DHEAS) levels in RA patients on and off steroid treatments

Steroid administration	No. of cases	ACTH (pg/ml)	DHEAS (ng/ml)
No	45	27.92 ± 19.74	883.91 ± 655.23
Yes	78	11.79 ± 10.13	418.12 ± 442.83

Values are mean ± SD
$P < 0.0001$

Table 3. ACTH levels in RA patients according to prednisolone dose

Dose (mg)	No. of cases	Mean (pg/ml) ± SD	P[a]
0	45	27.92 ± 19.74	
2.5	16	13.31 ± 8.50	0.0004*
5	47	11.51 ± 8.47	<0.0001*
7.5	9	7.67 ± 4.56	0.0003*
10	6	16.17 ± 24.70	0.0514

[a] Adjusted for age and sex
* Significant difference compared with 0 mg dose

Table 4. DHEAS levels in RA patients according to prednisolone dose

Dose (mg)	No. of cases	Mean (ng/ml) ± SD	P^a
0	45	883.91 ± 655.23	
2.5	16	443.94 ± 369.54	0.1648
5	47	482.43 ± 503.51	0.0002*
7.5	9	196.11 ± 173.38	0.0002*
10	6	178.50 ± 176.39	0.0271*

[a] Adjusted for age and sex
* Significant difference compared with 0 mg dose

Changes in ACTH and DHEAS levels in 14 patients for whom these parameters were measured twice

In one of the 14 patients, a steroid-free treatment approach was switched to prednisolone therapy at a dose of 5 mg/day. In another patient, the daily steroid dose was increased from 5 to 7.5 mg. In both of these patients, DHEAS levels decreased (Fig. 1). Some of the remaining 12 patients showed changes in ACTH and DHEAS levels, despite the constant steroid dosage (Fig. 2).

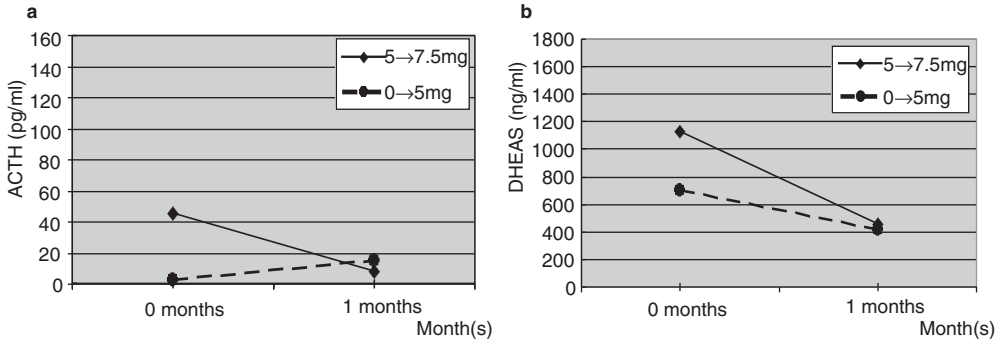

Fig. 1. Changes in adrenocorticotropic hormone (*ACTH*) and dehydroepiandrosterone sulfate (*DHEAS*) levels after increased doses of prednisolone in two patients

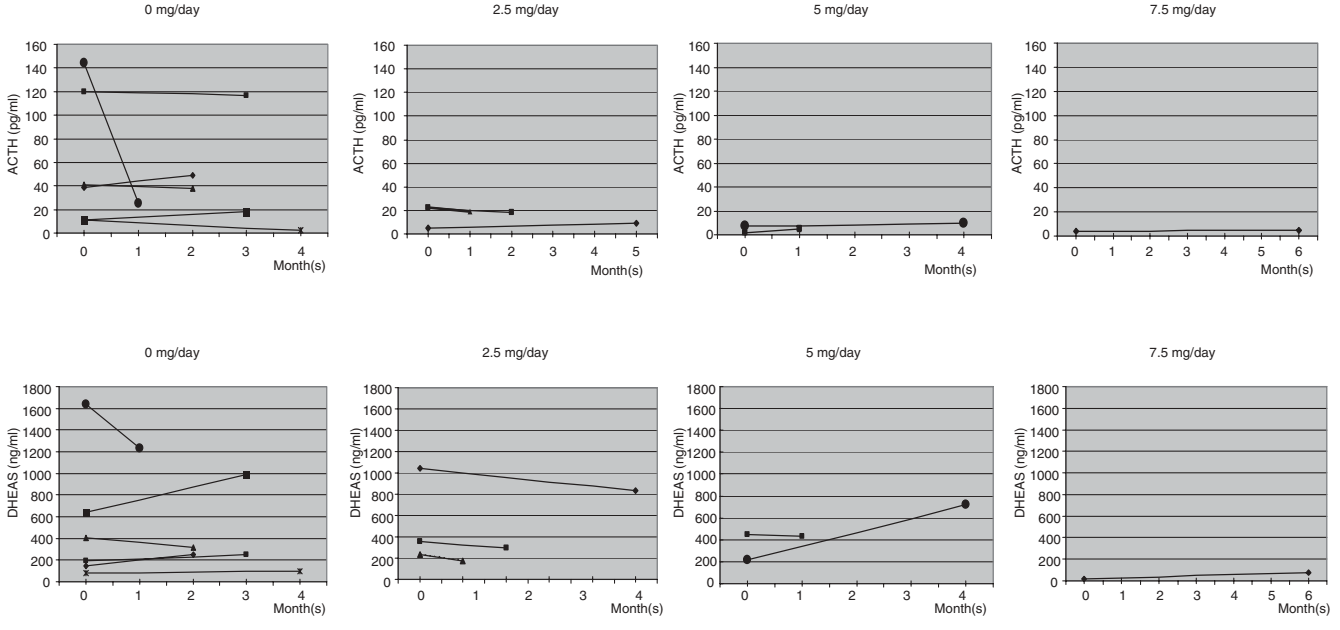

Fig. 2. Changes in adrenocorticotropic hormone (*ACTH*) and dehydroepiandrosterone sulfate (*DHEAS*) levels in patients on fixed doses of steroids

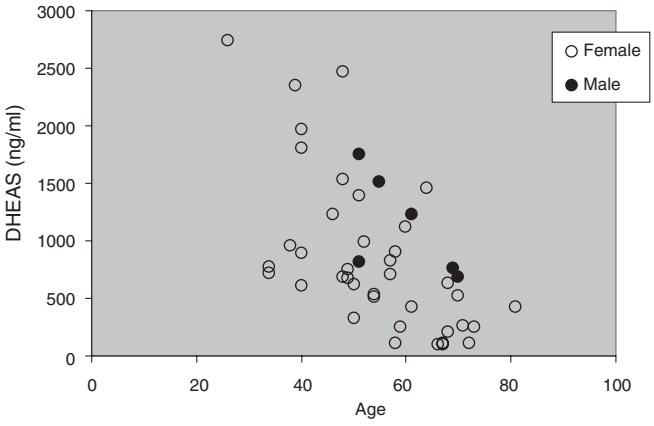

Fig. 3. Dehydroepiandrosterone sulfate (*DHEAS*) levels in rheumatoid arthritis patients without steroid treatment

Correlations of ACTH and DHEAS with age, ESR, CRP, MMP_3, and RBC values

We observed a correlation between ACTH and DHEAS ($r = 0.37125$). Dehydroepiandrosterone sulfate was negatively correlated with age ($r = -0.43464$). In addition, CRP values were correlated with ESR ($r = 0.50421$), RF ($r = 0.46516$), and MMP_3 ($r = 0.39224$) ($r > 0.3000$ or more was extracted).

Discussion

Dehydroepiandrosterone sulfate levels decrease with age,[2,3] and a high blood DHEAS level is related to a long life span.[12] It is also thought that DHEAS may exhibit anti-aging actions. In our patients, DHEAS was negatively correlated with age, supporting that supposition. Furthermore, cortisol and DHEAS are secreted by the adrenal cortex as a result of stimuli from ACTH secreted by the pituitary gland.[1] However, cortisol has a negative feedback mechanism, and blood levels of cortisol show a marked circadian variation.[1,13] In addition, steroids are administered to a high percentage of patients with RA. In comparison to cortisol, DHEAS does not have any negative feedback mechanism, and there may be less marked circadian variations,[1] meaning that the measurement of ACTH and DHEAS levels may be more useful for evaluating adrenal function.

Studies have reported that the blood levels of DHEAS in patients with RA are lower,[6–8] or similar[9] to those of normal controls; however, in the Hall et al.[7] study involving a large number of samples, it was reported that blood levels of DHEAS are lower in patients with RA, and that these levels are further reduced in patients receiving steroids or in those with a history of steroid use. We found that the levels of DHEAS and ACTH were decreased in the group receiving steroid therapy. There were no steroid dose-related differences in ACTH levels. However, DHEAS levels were reduced at a prednisolone dose of 2.5 mg/day, with levels significantly lower at a dose of 5 mg/day, and with further decreases at doses of 7.5 mg/day or higher. We consider a negative feedback mechanism to be involved in steroid therapy-related decreases in the levels of DHEAS and ACTH. While we observed significant differences in levels of DHEAS, there were no significant differences in ACTH levels related to steroid dose, possibly because there was circadian variation in ACTH[1] and because samples were not collected at a specific time point (samples were collected between 08:30 and 11:00 h). Another study has reported that steroid therapy reduces the responsiveness of ACTH to hypoglycemic stress stimuli, although the function of ACTH is maintained.[14] Many of our subjects visited our hospital as outpatients from distant areas by train or bus, which may have influenced ACTH levels as a result of physical activity.[15] In addition, mental stress and increases in interleukin-6 and tumor necrosis factor-α may influence the ACTH level.[1,10] These factors may have contributed to no steroid dose related differences being noted in the ACTH levels, which differed from our results for DHEAS levels.

A previous study has reported that blood DHEAS levels are constant during clinical courses.[8] However, in our study, some of our patients showed changes in ACTH and DHEAS levels, although the steroid dosage was not changed. This may be related to physical or mental stressors, including increased RA activity.[10,16–19] Concerning the relationship between stress and hormones, Nishikaze and colleagues hypothesized that urinary 17-hydroxycorticosteroid (17OHCS mg/g Cr.) and 17-ketosteroid sulfates (17KS-S mg/g Cr.), respectively, reflect the level of stress and recovery ability, and reported that the level of stress and the ability to cope with stress can be assessed by measuring these two urinary hormones.[20–22] Briefly, 17OHCS levels increase in the alarm reaction and resistance phases of Syle's general adaptation syndrome.[23] In the exhaustion phase, the 17OHCS and 17KS-S levels decrease. In the recovery phase, the 17KS-S level increases. We previously reported that the level of stress could be evaluated by measuring urinary levels of 17OHCS and 17KS-S in patients with RA.[24] Urinary 17OHCS is a metabolite of blood cortisol, and 17KS-S is a metabolite of blood DHEA(S).[20] In the presence of ACTH stimulation, cortisol is secreted by the adrenal cortex. We consider that the level of stress in patients with RA and their recovery ability can be evaluated by measuring ACTH and DHEAS levels, and urinary levels of 17OHCS and 17KS-S. Based on Syle's general adaptation syndrome, it is considered that the levels of ACTH and cortisol may increase in the alarm reaction and resistance phases. However, we speculate that when ACTH stimulation shifts to cortisol in these phases, there may be no change or a reduction in DHEAS levels.[9,25] In the exhaustion phase, the adrenal gland function appears to become inhibited, with cortisol and DHEA levels decreasing despite an increase in ACTH levels. In addition, when functional disorders of the hypothalamic-pituitary-adrenocortical (HPA) system occur, the levels of ACTH and DHEAS decrease.[26] Increases in ACTH and decreases in DHEAS were observed in cases of adrenal hypofunction.[1] In this study, as was reported by Hall et al.,[19] DHEAS was not

correlated with ESR or CRP. However, some studies have found correlations between DHEAS and these parameters.[27,28] We speculate that DHEAS levels are increased in patients with marked inflammatory responses, involving ESR and CRP, in the resistance phase described by Syle.[9,10] Thus, the measurement of ACTH and DHEAS may be useful for evaluating HPA system function and stress.

An association between blood levels of DHEAS and osteoporosis has also been suggested,[4,29,30] and it has been suggested that DHEA intake helps prevent osteoporosis.[31–33] On the other hand, it has been reported that prednisolone at doses of more than 5 mg/day may increase the risk of osteoporosis.[34,35] This was supported by the results of our study. In short, DHEAS levels in patients receiving prednisolone at 7.5 mg/day or higher are lower than in those who receive 5 mg/day, thus increasing the risk of osteoporosis.[36] We therefore consider DHEAS may be a possible blood marker of osteoporosis in patients with RA. Dehydroepiandrosterone sulfate levels in the 45 patients not on steroid therapy are presented in Fig. 3. There was a marked variation in DHEAS levels. In patients with decreases in DHEAS, adrenal function may have been reduced. In this group with decreases in the DHEAS levels, adrenal hypofunction may have been associated with the various factors described above. Prior to steroid therapy, adrenal function in patients with RA should be evaluated by measuring blood levels of ACTH and DHEAS.

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