Complimentary and personal copy for
Sema Ciftci , Ozlem Soyluk, Alev Selek, Selvinaz Erol, Zeliha Hekimsoy, Ayse Esen, Huseyin Dursun, Serdar Sahin, Gonca Oruk8, Meral Mert, et al.



www.thieme.com

The Importance of DHEA-S Levels in Cushing's Syndrome; Is There a Cut-off Value in the Differential Diagnosis?

DOI 10.1055/a-1783-7901
Horm Metab Res 2022; 54: 232–237

This electronic reprint is provided for non-commercial and personal use only: this reprint may be forwarded to individual colleagues or may be used on the author's homepage. This reprint is not provided for distribution in repositories, including social and scientific networks and platforms.

Publisher and Copyright:
© 2022 . Thieme. All rights reserved.
Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany
ISSN 0018-5043

Reprint with the permission by the publisher only

Thieme

Thieme

The Importance of DHEA-S Levels in Cushing's Syndrome; Is There a Cut-off Value in the Differential Diagnosis?

Authors

Sema Ciftci[1], Ozlem Soyluk[2], Alev Selek[3], Selvinaz Erol[4], Zeliha Hekimsoy[5], Ayse Esen[1], Huseyin Dursun[6], Serdar Sahin[7], Gonca Oruk[8], Meral Mert[1], Huseyin Soylu[9], Banu Sarer Yurekli[10], Melek Eda Ertorer[11], Tulay Omma[12], Mehtap Evran[13], Mine Adas[14], Seher Tanrikulu[15], Kadriye Aydin[16], Zafer Pekkolay[17], Bülent Can[18], Ersen Karakilic[19], Zuleyha Karaca[6], Habib Bilen[20], Zeynep Canturk[3], Berrin Cetinarslan[3], Pinar Kadioglu[7], Sema Yarman[2]

Affiliations

1 Department of Endocrinology and Metabolsim, University of Health Sciences, Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Istanbul, Turkey
2 Department of Endocrinology and Metabolism, Istanbul University, Fatih, Turkey
3 Department of Endocrinology and Metabolism, Kocaeli University, Kocaeli, Turkey
4 Department of Endocrinology and Metabolism, University of Health Sciences, Sisli Hamidiye Etfal Training and Research Hospital, Istanbul, Turkey
5 Endocrinology, Celal Bayar University, Manisa, Turkey
6 Department of Endocrinology and Meatbolism, Erciyes University School of Medicine, Kayseri, Turkey
7 Department of Endocrinology and Metabolism, Istanbul University Cerrahpasa Faculty of Medicine, Istanbul, Turkey
8 Department of Endocrinology and Metabolism, Izmir Ataturk Training and Research Hospital, Izmir, Turkey
9 Department of Endocrinology and Metabolism, Ondokuz Mayis University, School of Medicine, Samsun, Turkey
10 Department of Endocrinology and Metabolism, Ege University Faculty of Medicine, Izmir, Turkey
11 Department of Endocrinology and Metabolism, Baskent University Faculty of Medicine, Adana, Turkey
12 Department of Endocrinology and Metabolism, Ankara Training and Research Hospital, Ankara, Turkey
13 Department of Endocrinology and Metabolism, Çukurova University Medical Faculty, Adana, Turkey
14 Department of Endocrinology and Metabolism, University of Health Sciences, Okmeydani Training and Research Hospital, Istanbul, Turkey
15 Department of Endocrinology and Metabolsim, Haydarpaşa Numune Training and Research Hospital, Istanbul, Turkey
16 Department of Endocrinology and Metabolism, Kartal Dr. Lutfi Kirdar Training and Research Hospital, Istanbul, Turkey
17 Department of Endocrinology and Metabolism, Dicle Faculty of Medicine, Diyarbakır, Turkey
18 Department of Endocrinology and Metabolism, Istanbul Medeniyet University Faculty of Medicine, İstanbul, Turkey
19 Department of Endocrinology and Metabolism, Canakkale Onsekiz Mart University Medical Faculty, Çanakkale, Turkey
20 Department of Endocrinology and Metabolism, Ataturk University Faculty of Medicine, Ezurum, Turkey

Key words

Cushing's disease, adrenal Cushing's syndrome, ectopic ACTH syndrome, dehydroepiandrosterone-sulfate, DHEA-S cut-off, differential diagnosis

received 09.01.2022
accepted after revision 22.02.2022

Bibliography

Horm Metab Res 2022; 54: 232–237
DOI 10.1055/a-1783-7901
ISSN 0018-5043
© 2022. Thieme. All rights reserved.
Georg Thieme Verlag, Rüdigerstraße 14, 70469 Stuttgart, Germany

Correspondence

Dr. Sema Ciftci
University of Health Sciences
Bakirkoy Dr. Sadi Konuk Training and Research Hospital
Department of Endocrinology and Metabolsim
Bakırköy
34190 Istanbul
Turkey
Tel.: + 905325940967, Fax: + 902124147171
sdogansen@gmail.com

ABSTRACT

The purpose of this study was to determine possible cut-off levels of basal DHEA-S percentile rank in the differential diagnosis of patients with Cushing's syndrome (CS) with ACTH levels in the gray zone and normal DHEA-S levels. In this retrospective study including 623 pathologically confirmed CS, the DHEA-S percentile rank was calculated in 389 patients with DHEA-S levels within reference interval. The patients were classified as group 1 (n = 265 Cushing's disease; CD), group 2 (n = 104 adrenal CS) and group 3 (n = 20 ectopic ACTH syn-

Electronic reprint for personal use

drome).ROC-curve analyses were used to calculate the optimal cut-off level of DHEA-S percentile rank in the reference interval in the differential diagnosis of CS, and the effectiveness of this cut-off level in the identification of the accurate etiology of CS was assessed in patients who were in gray zone according to their ACTH levels. The DHEA-S percentile rank in the reference interval were significantly lower in group 2 compared to the other two groups ($p < 0.001$), while group 1 and group 3 had similar levels. The optimal cut-off level of DHEA-S percentile rank in the reference interval providing differential diagnosis between group 1 and group 2 was calculated as 19.5th percentile (80.8 % sensitivity, 81.5 % specificity) and the level demonstrated the accurate etiology in 100 % of CD and 76 % of adrenal CS patients who were in the gray zone. This study showed that the cut-off value of DHEA-S level less than 20 % of the reference interval could be used for differential diagnosis of CD and adrenal CS with high sensitivity and specificity, and it should be taken into the initial evaluation.

Introduction

Cushing's syndrome (CS) is an unusual disorder characterized by excessive cortisol secretion [1, 2]. The early diagnosis and treatment of CS are quite important since untreated hypercortisolism is associated with increased morbidity and mortality [2–4]. Although there are significant advances in the pathophysiology and treatment modalities, there are still many challenges during the evaluation of CS including the differential diagnosis of the exact etiology [5, 6]. In the differential diagnosis of CS, depending on the laboratory analysis, 80 % of CS are adrenocorticotropic hormone (ACTH) dependent and the remaining 20 % are ACTH independent [6, 7]. The discrimination of ACTH dependent and independent CS is clear when the ACTH level is > 20 pg/ml or < 10 pg/ml, respectively [1, 3, 7]. However, ACTH levels between 10–20 pg/ml are called the gray zone, and there are some CS patients whose ACTH level is within this range [1, 3, 7]. For the discrimination of the exact etiology in these patients, dynamic tests like high-dose dexamethasone suppression test (HDDST), corticotropin-releasing hormone (CRH) or desmopressin stimulation test, and the invasive procedure, inferior petrosal sinus sampling (IPSS), which is the gold standard test are used [3, 8].

The synthesis and secretion of dehydroepiandrosterone sulfate (DHEA-S), a steroid hormone from adrenal glands is dependent on ACTH [9]. This characteristic enables the evaluation of ACTH secretion by DHEA-S levels and the DHEA-S levels are expected to be suppressed in adrenal CS and increased in ACTH dependent CS [10]. However, in many studies, serum DHEA-S levels have been reported mostly within the reference interval contrary to expected in ACTH dependent CS, and it has been reported also as low or within the reference interval in adrenal CS [11–17]. The data suggest that ACTH alone does not control adrenal androgen secretion. ACTH precursor molecule pro-opiomelanocortin (POMC), and factors like β-endorphin, β-lipotrophin are thought to be related to secretion of DHEA-S [9, 15, 18, 19].

The utility of the DHEA-S level in the differential diagnosis of a small number of patients with CS evaluated in the literature [11–16]. In an early study of these, the authors concluded that the determination of DHEA-S levels is useful in the differential diagnosis of CS since the plasma DHEA-S levels are fairly stable [11]. On the other hand, recently a retrospective multicenter study assessed the utility of plasma ACTH to differentiate adrenal CS from CD and compare it with that of the HDDST results and serum DHEA-S levels. In that study, the serum DHEA-S levels demonstrated a wide range of overlap between patients with adrenal CS and those with CD, and the diagnostic value of DHEA-S was reported to be poor [13]. In a few studies comparing DHEA-S levels in patients with EAS and CD, DHEA-S levels have been shown to be not useful for the differential diagnosis of CS [12, 14, 15].

Although suppressed or high DHEA-S levels guide us in the differential diagnosis of CS, a cut-off levels of basal DHEA-S percentile rank to determine this distinction for patients with DHEA-S levels in the reference interval as observed in most of the cases has not been studied. This study aims to determine the differential diagnostic value of basal DHEA-S levels compared to other tests in CS and to determine the cut-off levels of basal DHEAS percentile rank in the reference interval in the differential diagnosis of a larger number patient with pathologically confirmed CS.

Patients and Methods

Patients

This is a retrospective multicenter study. The data of 623 patients (524 female/99 male) with pathologically confirmed CS who were admitted to 20 tertiary centers and diagnosed after 2000 in Turkey are collected. All the data of patients gathered from these different centers were standardized.

The diagnosis and differential diagnosis of CS were confirmed according to the typical clinical signs and symptoms of hypercortisolemia, radiological imaging findings, basal hormonal evaluations such as cortisol, ACTH, DHEA-S, low-dose DST (LDDST), HDDST, and 24-hour urinary free cortisol (24h-UFC) or IPSS all with respect to the current guideline [2]. And also, the confirmation of the diagnosis was made by the development of postsurgical adrenal insufficiency for ACTH dependent and independent CS. ACTH immunostaining in postsurgical pathological specimens is used for detecting ACTH dependent CS (CD and EAS), and demonstration adenoma tissue after adrenalectomy is used for detecting ACTH independent CS. Accordingly, 353 patients were diagnosed with CD, 242 patients with adrenal CS, and 28 patients with EAS. Patients with cyclic CS, subclinical CS patients with cortisol levels < 5 µg/dl in LDDST or patients who did not undergo surgery and patients with the diagnosis of adrenal carcinoma are excluded from the study. Additionally, in order to assess the role of DHEA-S in differential diagnosis, we excluded the patients with suppressed DHEA-S levels as expected in patients with adrenal CS, and increased DHEA-S levels as expected in patients with ACTH-dependent CS.

Electronic reprint for personal use

Assays and Hormonal Evaluation

The assessment methods, measurement units, and reference ranges of all hormone levels were obtained from all centers. Measuring basal morning DHEA-S, cortisol and ACTH, 24h-UFC, and DSTs at least once preoperatively was a prerequisite for inclusion in the study. However, up to three hormone levels measured at different times were requested and the mean of them was included. The methods of serum DHEA-S measurement were electrochemiluminescence immunoassay and high-performance liquid chromatography-tandem mass spectrometry. For DHEA-S levels in their normal reference range (age and sex-matched), the DHEA-S percentile rank was calculated with the formula as follows: [(calculated DHEA-S levels – lower limit of DHEA-S reference interval)/(upper limit of DHEA-S reference interval – lower limit of DHEA-S reference interval)] × 100. Thus, the percentile rank values represent the percentage of DHEA-S levels at the lower and upper bound of that normal reference range. 24h-UFC levels were measured by high-performance liquid chromatography, competitive binding immunoenzymatic assay, chemiluminescence. 24h-UFC ratios were calculated by dividing the 24h-UFC levels to the upper limit of the respective reference range for all patients. Blood samples were collected from patients in accordance with bedside blood collection protocol and, serum cortisol and ACTH levels were measured by using an automated electrochemiluminescent immunoassay.

Design and Procedures

Based on ACTH levels used in the differential diagnosis of CS; ACTH < 10 pg/ml was accepted as ACTH-independent, ACTH > 20 pg/ml as ACTH-dependent, and ACTH between 10–20 pg/ml as gray zone [1, 3, 7]. For patients with CD (n = 353) and patients with EAS (n = 28), the DHEA-S level in the reference interval and elevated according to age and gender, and for patients with adrenal CS (n = 242), the DHEA-S level in the reference interval (for age and gender) and suppressed were all recorded.

A total of 389 patients with DHEA-S levels within the reference interval were divided into groups according to their differential diagnosis. Accordingly, those with a diagnosis of CD (n = 265) were classified as group 1, those with adrenal CS (n = 104) as group 2, and those with a diagnosis of EAS (n = 20) as group 3. Groups were compared with each other according to age at diagnosis, sex, the estimated duration of CS (the estimated duration from the beginning of symptoms until the diagnosis of CS according to the disease history expressed by the patient), levels of basal cortisol, ACTH, DHEA-S percentile rank in the reference interval, and 24h-UFC range, LDDST and HDDST results.

Possible cut-off values for DHEA-S percentile rank in the reference interval were evaluated to make a differentiation between groups 1 and 2, groups 2 and 3, and groups 1 and 3. And, the effectiveness of the cut-off level in the identification of the accurate etiology of CS was assessed in patients who were in the gray zone according to their ACTH levels.

Statistical analyses

Statistical analyses were performed using SPSS version 24.0. Categorical variables were defined by frequency and percentage rate, and continuous variables were defined by mean ± standard deviation (SD). Kolmogorov-Smirnov and Shapiro-Wilk normality tests were used for every variable to determine the analytic method for testing of hypotheses. In the comparison of triple groups, the Kruskal-Wallis test was performed due to the finding of non-normally distribution of variables. The mean rank was evaluated for the source of difference. Statistically significant results were defined with a $p < 0.05$. The areas under the curves (AUCs) were calculated by receiver operating characteristic (ROC) analysis to determine the cut-off thresholds for DHEA-S percentile rank in the reference interval. The procedure was used to estimate the 95 % CIs of the AUCs and to test for differences between the areas of particular curves. Sensitivity and specificity calculations were made to determine the power of the DHEA-S levels for discriminations of groups.

The study was approved by the Clinical Ethics Committee of Bakirkoy Dr. Sadi Konuk Training and Research Hospital. This study was performed in accordance with the Helsinki recommendations.

Results

The mean age at diagnosis of 623 patients was 42 ± 12 years. Distribution of ACTH and DHEA-S levels in the CD, adrenal CS, EAS patients are given in ▶ **Table 1**.

By comparison of the groups, group 1 patients were younger than the group 2 patients ($p < 0.001$), while group 3 included more male patients than the other two groups ($p < 0.001$). The estimated duration of CS was similar in all groups. Basal cortisol, ACTH, 24h-UFC ratios, and HDDST results were significantly different between the three groups (group 3 > group 1 > group 2), ($p < 0.001$, for all comparisons). LDDST results of group 3 were significantly higher than the other two groups ($p < 0.001$). The DHEA-S percentile rank in the reference interval was significantly lower in group 2 compared to the other two groups ($p < 0.001$), while group 1 and group 3 had similar DHEA-S percentile rank in the reference interval. The comprehensive data about the comparison of groups are given in ▶ **Table 2**.

The optimal cut-off level of DHEA-S percentile rank in the reference interval with the highest sensitivity of 80.8 % and specificity of 81.5 % calculated by ROC curves for group 1 and group 2 was determined as 19.5th percentile with an AUC of 0.89 (95 % CI: 0.85–0.92) (▶ **Fig. 1** ROC curve). The cut-off level of DHEA-S percentile rank in the reference interval the accurate etiology in 100 % of 12 CD and 76 % of 25 adrenal CS patients who were in the gray zone according to their ACTH levels. The optimal cut-off level of DHEA-S percentile rank in the reference interval with the highest sensitivity of 95 % and specificity of 87.5 % calculated by ROC curves for group 2 and group 3 was determined as 23.5 with an AUC of 0.96 (95 % CI: 0.93–0.99) (▶ **Fig. 2** ROC curve). No significant the optimal cut-off level of DHEA-S percentile rank in the reference interval was found between group 1 and group 3.

Discussion

In this study, we have shown that the DHEAS cut-off level is quite useful in differentiating adrenal and pituitary CS, especially in patients who are in the gray zone according to ACTH level. Although DHEA-S is not one of the recommended tests in the initial diagnos-

Electronic reprint for personal use

▶ **Table 1** Distribution of ACTH and DHEAS levels in Cushing's disease, adrenal Cushing's syndrome, and ectopic ACTH syndrome.

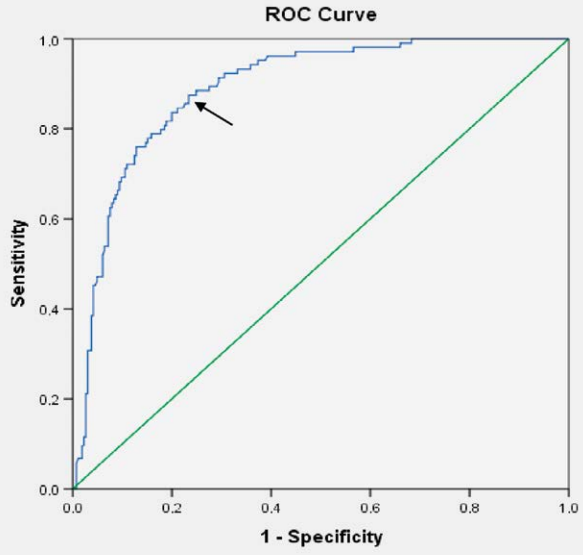
n = 623	Cushing's disease (n = 353)		Adrenal Cushing's syndrome (n = 242)		Ectopic ACTH syndrome (n = 28)	
	ACTH levels > 20 pg/ml (n = 339)	ACTH levels 10–20 pg/ml (n = 14)	ACTH levels < 10 pg/ml (n = 198)	ACTH levels 10–20 pg/ml (n = 44)	ACTH levels > 20 pg/ml (n = 28)	ACTH levels 10–20 pg/ml (n = 0)
Suppressed DHEA-S levels (n; %)	–	–	119 (60)	19 (43)	–	–
DHEA-S in the normal range (n; %)	253 (75)	12 (86)	79 (40)	25 (57)	20 (71 %)	–
Elevated DHEA-S levels (n; %)	86 (25)	2 (14)	–	–	8 (29 %)	–

ACTH: Adrenocorticotropic hormone; DHEA-S: Dehydroepiandrosterone sulfate.

▶ **Table 2** Comparison of groups in terms of baseline characteristics.

n = 389	Group 1 (Cushing's disease) n = 265	Group 2 (Adrenal CS) n = 104	Group 3 (EAS) n = 20	p
Age at diagnosis (years) mean ± SD	40 ± 12	48 ± 11	43 ± 14	**< 0.001**[a]
Sex (F/M)	230/35	89/15	13/7	**0.029**[b]
The estimated duration of CS (months) mean ± SD	23 ± 26	19 ± 18	10 ± 4	NS
Basal cortisol (µg/dl) mean ± SD	23 ± 8	17 ± 6	35 ± 19	**< 0.001**[c]
Basal ACTH (pg/ml) mean ± SD	69 ± 41	7.7 ± 4.3	162 ± 254	**< 0.001**[c]
Basal DHEA-S percentile rank in the normal range (µg/dl) mean ± SD	47 ± 27	12 ± 12	56 ± 25	**< 0.001**[d]
Cortisol level after LDDST (µg/dl) mean ± SD	13.5 ± 7.2	13 ± 6.2	26.2 ± 14.5	**< 0.001**[b]
Cortisol level after HDDST (µg/dl) mean ± SD	5.6 ± 5.9	11 ± 8	27.9 ± 25	**< 0.001**[c]
24h-UFC ratio mean ± SD	2.8 ± 8	1.2 ± 1.8	37 ± 96	**0.03**[e]

$p < 0.05$ statistically significant. Significant p-values are shown in bold; [a] Statistically significant between group 1 & group 2; [b] Statistically significant between group 1 & group 3, group 2 & group 3; [c] Statistically significant between group 1 & group 2, group 2 & group 3, group 1 & group 3; [d] Statistically significant between group 1 & group 2, group 2 & group 3; [e] Statistically significant between group 2 & group 3; NS: Not significant; ACTH: Adrenocorticotropic hormone; CS: Cushing's syndrome; CD: Cushing's disease; DHEA-S: dehydroepiandrosterone sulfate; EAS: Ectopic ACTH syndrome; HDDST: High-dose dexamethasone suppression test; LDDST: Low-dose dexamethasone suppression test; UFC: Urinary free cortisol.

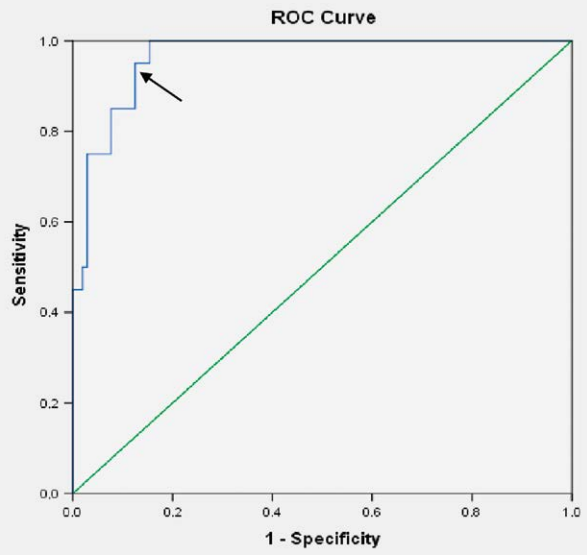


▶ **Fig. 1** The arrow on the ROC curve indicates the cut-off level of DHEA-S percentile rank in the normal range with the highest sensitivity of 80.8 % and specificity of 81.5 % for group 1 and group 2 which was determinated as 19.5th percentile; AUC: 0.89 (95 % CI: 0.8–0.92).

tic evaluation of CS, it is well known that suppressed DHEA-S levels support the diagnosis of adrenal CS [2, 5, 10]. In our cohort, DHEA-S levels were also suppressed in more than half of adrenal CS patients. The half-life of DHEA-S, which is formed by sulfation of DHEA, thus increases to 10 hours and remains at this level without daily variations throughout the day, ensuring its reliability [10, 20].

The synthesis and secretion of DHEA-S are dependent mainly on ACTH, and this is also recently shown in a study by the measurement of adrenal vein hormones before and after stimulation with ACTH [11, 21]. In our cohort, DHEA-S percentile rank was significantly lower in ACTH-independent CS patients than the other two groups in those with normal DHEA-S levels. However, in our study, the DHEA-S percentile rank in the reference interval were similar in CD and EAS groups. Despite this similarity in patients with

Electronic reprint for personal use



▶ **Fig. 2** The arrow on the ROC curve indicates the cut-off level of DHEA-S percentile rank in the normal range with the highest sensitivity of 95% and specificity of 87.5% for group 2 and group 3 which was determinated as 23.5th percentile; AUC: 0.96 (95% CI: 0.93–0.99).

ACTH-dependent CS, basal cortisol levels were significantly different in these groups. This difference could be a result of the variability in the processing of POMC occurring in CD and EAS may cause differences between cortisol and androgen levels in these patients, as Cunningham et al. [15] stated in their study.

DHEA-S levels in CS has quite an attention in the past, however, it has lost old interest especially in the evaluation of CS [11, 12, 14–16, 22]. Therefore, we wanted to draw attention again to the importance of DHEA-S in the differential diagnosis of CS. The etiology of CS is clear in case of high ACTH level with an obvious pituitary adenoma, or in case of adrenal adenoma with suppressed ACTH level, or in CS with very high ACTH level, hypokalemia and demonstration of a neuroendocrine tumor with normal pituitary imaging [2, 3, 8]. Among the dynamic tests such as HDDST, CRH, and desmopressin stimulation tests used in cases where the etiology is not clear are gradually losing their importance due to low accuracy and these tests are recommended to be performed only when IPSS is not possible [23]. However, it is valuable to be able to make differential diagnosis without IPSS, as this is an invasive procedure and should be applied in experienced centers. Therefore, there are always studies of investigating for differential diagnostic tests, and in one of these studies, the researchers evaluated the ACTH/cortisol ratio, and a ratio above 2.5 was reported to be specific for CD [24].

In our study, in order to understand the importance of DHEA-S in differential diagnosis, we excluded patients with suppressed levels as expected in patients with adrenal CS, and patients with high DHEA-S levels in those with ACTH-dependent CS. We tried to find a cut-off value for DHEA-S percentile rank, especially in those with DHEA-S values within the reference interval. With the DHEA-S percentile rank in the reference interval, we have created a homogenization for DHEA-S levels studied from different centers with different methods. In our study, the cut-off value for DHEA-S percentile rank in the reference interval which we found for differential diagnosis of CD and adrenal CS is DHEA-S percentile rank in the reference interval with high sensitivity. Accordingly, in our study, it was shown that those below the 20th percentile of the reference interval indicated adrenal etiology, and those above the 20th percentile of the reference interval indicated CD. In contrast to our study, which is a smaller number of patients containing, DHEA-S levels were significantly different between adrenal CS and CD but there was too much overlap between the groups [13]. Moreover, the cut-off value of DHEA-S percentile rank in the reference interval also demonstrated the accurate etiology in 100% of CD and 76% of adrenal CS patients who were in the gray zone in terms of ACTH levels. The diagnostic accuracy rate of this cut-off level was found to be quite high in a small number of patients whose ACTH level remained in the gray zone in this study which included many more patients compared to the literature. We also found a cut-off DHEA-S percentile rank in the reference interval at 23.5th percentile to differentiate the rare etiology of EAS from adrenal CS. Usually, it is not difficult to discriminate EAS and adrenal CS, but in case of subclinical CS when the ACTH is not significantly suppressed there may be some difficulties. However, for a definite conclusion, more studies including patients with the similar conditions are needed.

One of the most challenging issues in the evaluation of CS is the differential diagnosis of EAS and CD. Because both are ACTH dependent CS and it is not always possible to demonstrate a visible pituitary or neuroendocrine tumor, and the invasive procedure IPSS is often required for the detection of etiology [23]. Therefore, to find a cut-off level of DHEA-S percentile rank in the reference interval which would discriminate between them is particularly important. However, both EAS and CD groups had similar DHEA-S percentile rank in the reference interval in our study. Although it was the most comprehensive study on this issue, the number of patients with EAS was small, perhaps a cut-off value could be found by increasing the number of these patients. There were few studies with lower patient numbers evaluating the DHEA-S level in EAS and CD patients, similarly our study, they showed that there was no significant difference between the groups [12, 14, 15, 25, 26]. Therefore, invasive tests still maintain their importance for the differentiation of EAS and CD.

In the literature the course of postoperative DHEA-S and cortisol levels in patients with CS were also evaluated and DHEA-S levels were shown to improve in a longer time in contrast to cortisol levels after pituitary or adrenal surgery [12, 16, 17, 27]. The most likely explanation for this is the significant and long-lasting suppression of zona reticularis due to insufficient ACTH secretion because cells of zona reticularis are thought to contain the ACTH-dependent enzyme 17,20 desmolase abundantly [16, 28]. DHEA-S levels in the postoperative period were also evaluated for their predictive value of remission in CD and in some studies the percentage of decrease in DHEA-S levels was shown to be associated with persistent or recurrent disease [25, 29, 30]. Since the retrospective design of our study and not available of consecutive postoperative DHEA-S levels in many patients, we could not evaluate the course and changes of DHEA-S levels in the postoperative period.

The most important limitation of our study is that hormonal data including DHEA-S levels were assessed retrospectively from

Electronic reprint for personal use

different centers and different periods. However, calculation of DHEA-S percentile rank in the reference interval eliminated these heterogeneities. And also, in a rare disease like CS, the achievement of the pathologically confirmed CS patient numbers as in our study is very difficult for a single-center and logical determination of cut-off values could be possible by including a large number of patients. Due to the low prevalence of EAS, another limitation of this study is the cut-off level of DHEA-S percentile rank in the reference interval could not be determined in the differential diagnosis between CD and a small number of EAS.

As a result, although DHEA-S levels change in correlation with ACTH in CS, the cut-off level of DHEA-S percentile rank for DHEA-S levels within the reference interval may be useful especially in the differential diagnosis of CD and adrenal CS patients who are in the gray zone according to their ACTH levels. Similarly, cut-off values of DHEA-S found for discrimination of EAS and adrenal CS also have very high sensitivity and specificity. Since the plasma DHEA-S levels are fairly stable and easily measurable, all these data need to be supported with further studies.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Orth DN. Cushing's syndrome. N Engl J Med 1995; 332: 791–803

[2] Nieman LK, Biller BM, Findling JW et al. The diagnosis of Cushing's syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2008; 93: 1526–1540

[3] Arnaldi G, Angeli A, Atkinson AB et al. Diagnosis and complications of Cushing's syndrome: a consensus statement. J Clin Endocrinol Metab 2003; 88: 5593–5602

[4] Nieman LK, Biller MB, Findling JW et al Endocrine Society. Treatment of Cushing's syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2015; 100: 2807–2831

[5] Nieman LK. Diagnosis of Cushing's syndrome in the modern era. Endocrinol Metab Clin North Am 2018; 47: 259–273

[6] Lacroix A, Feelders RA, Stratakis CA et al. Cushing's syndrome. Lancet 2015; 386: 913–927

[7] Newell-Price J, Trainer P, Besser GM et al. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr Rev 1998; 19: 647–667

[8] Lindsay JR, Nieman LK. Differential diagnosis and imaging in Cushing's syndrome. Endocrinol Metab Clin N Am 2005; 34: 403–421

[9] Parker LN, Odell WD. Control of adrenal androgen secretion. Endocr Rev 1980; 1: 392–410

[10] Arnaldi G, Martino M. Androgens in Cushing's syndrome. Front Horm Res 2019; 53: 77–91

[11] Yamaji T, Ibayashi H. Plasma dehydroepiandrosterone sulfate in normal and pathological conditions. J Clin Endocrinol Metab 1969; 29: 273–278

[12] Barbetta L, Dall'Asta C, Re T et al. Androgen secretion in ectopic ACTH syndrome and in Cushing's disease: modifications before and after surgery. Horm Metab Res 2001; 33: 596–601

[13] Hong AR, Kim JH, Hong ES et al. Limited diagnostic utility of plasma adrenocorticotropic hormone for differentiation between adrenal Cushing syndrome and Cushing disease. Endocrinol Metab (Seoul) 2015; 30: 297–304

[14] Hauffa BP, Kaplan SL, Grumbach MM. Dissociation between plasma adrenal androgens and cortisol in Cushing's disease and ectopic ACTH-producing tumour: relation to adrenarche. Lancet 1984; 1: 1373–1376

[15] Cunningham SK, McKenna TJ. Dissociation of adrenal androgen and cortisol secretion in Cushing's syndrome. Clin Endocrinol (Oxf) 1994; 41: 795–800

[16] Yamaji T, Ishibashi M, Sekihara H et al. Serum dehydroepiandrosterone sulfate in Cushing's syndrome. J Clin Endocrinol Metab 1984; 59: 1164–1168

[17] Kleiber H, Rey F, Temler E et al. Dissociated recovery of cortisol and dehydroepiandrosterone sulphate after treatment for Cushing's syndrome. J Endocrinol Invest 1991; 14: 489–492

[18] McKenna TJ, Cunningham SK. The control of adrenal androgen secretion. J Endocrinol 1991; 129: 1–3

[19] Genazzani AR, Facchinetti F, Petraglia F et al. Correlations between plasma levels of opioid peptides and adrenal androgens in prepuberty and puberty. J Steroid Biochem 1983; 19: 891–895

[20] Vaitukaitis IL, Dale SL, Melby JC. Role of ACTH in the secretion of dehydroepiandrosterone and its sulfate ester in man. J Clin Endocrinol Metab 1969; 29: 1443–1447

[21] Rege J, Nakamura Y, Satoh F et al. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. J Clin Endocrinol Metab 2013; 98: 1182–1188

[22] Horio S, Funahashi H, Mizuno S. Plasma steroid hormones in Cushing's syndrome: their relation to cause and clinical manifestations (Author's Transl). Nihon Naibunpi Gakkai Zasshi 1981; 57: 807–825

[23] Valassi E, Franz H, Brue T et al.ERCUSYN study group Diagnostic tests for Cushing's syndrome differ from published guidelines: data from ERCUSYN. Eur J Endocrinol 2017; 176: 613–624

[24] Selek A, Cetinarslan B, Canturk Z et al. The utility of preoperative ACTH/cortisol ratio for the diagnosis and prognosis of Cushing's disease. J Neurosci Rural Pract 2018; 9: 106–111

[25] Burkhardt T, Schmidt NO, Vettorazzi E et al. DHEA(S) – a novel marker in Cushing's disease. Acta Neurochir (Wien) 2013; 155: 479–484

[26] Wajchenberg BL, Mendonca BB, Liberman B et al. Ectopic adrenocorticotropic hormone syndrome. Endocr Rev 1994; 15: 752–787

[27] Nawata H, Higuchi K, Yanase T et al. Mechanism of dissociation of cortisol and adrenal androgen secretion after removal of adrenocortical adenoma in patients with Cushing's syndrome. Endocrinol Jpn 1985; 32: 691–700

[28] O'Hare MJ, Nice EC, Neville AM. Regulation of androgen secretion and sulfoconjugation in the adult human adrenal cortex: studies with primary monlayer cell cultures. In: Genazzani AR, Thijssen JHH, Siiteri PK (eds). Adrenal Androgens. Raven Press; New York: 1980: 7

[29] Kuo CH, Shih SR, Li HY et al. Adrenocorticotropic hormone levels before treatment predict recurrence of Cushing's disease. J Formos Med Assoc 2017; 116: 441–447

[30] El Asmar N, Rajpal A, Selman WR et al. The value of perioperative levels of ACTH, DHEA, and DHEA-S and tumor size in predicting recurrence of Cushing disease. J Clin Endocrinol Metab 2018; 103: 477–485

Electronic reprint for personal use