Adrenal Morphology as an Indicator of Long-Term Disease Control in Adults with Classic 21-Hydroxylase Deficiency

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Background: Monitoring adults with classical 21-hydroxylase deficiency (21OHD) is challenging due to variation in clinical and laboratory settings. Moreover, guidelines for adrenal imaging in 21OHD are not yet available. We evaluated the relationship between adrenal morphology and disease control status in classical 21OHD.

Methods: This retrospective, cross-sectional study included 90 adult 21OHD patients and 270 age- and sex-matched healthy controls. We assessed adrenal volume, width, and tumor presence using abdominal computed tomography and evaluated correlations of adrenal volume and width with hormonal status. We investigated the diagnostic performance of adrenal volume and width for identifying well-controlled status in 21OHD patients (17α-hydroxyprogesterone [17-OHP] <10 ng/mL).

Results: The adrenal morphology of 21OHD patients showed hypertrophy (45.6%), normal size (42.2%), and hypotrophy (12.2%). Adrenal tumors were detected in 12 patients (13.3%). The adrenal volume and width of 21OHD patients were significantly larger than those of controls (18.2 ± 12.2 mL vs. 7.1 ± 2.0 mL, 4.7 ± 1.9 mm vs. 3.3 ± 0.5 mm, $P < 0.001$ for both). The 17-OHP and androstenedione levels were highest in patients with adrenal hypertrophy, followed by those with normal adrenal glands and adrenal hypotrophy ($P < 0.05$ for both). Adrenal volume and width correlated positively with adrenocorticotropic hormone, 17-OHP, 11β-hydroxytestosterone, progesterone sulfate, and dehydroepiandrosterone sulfate in both sexes ($r = 0.33$–0.95, $P < 0.05$ for all). For identifying well-controlled patients, the optimal cut-off values of adrenal volume and width were 10.7 mL and 4 mm, respectively (area under the curve, 0.82–0.88; $P < 0.001$ for both).

Conclusion: Adrenal volume and width may be reliable quantitative parameters for monitoring patients with classical 21OHD.

Keywords: Adrenal hyperplasia, congenital; Adrenal cortex; Adrenal volume; Androgens

INTRODUCTION

Congenital adrenal hyperplasia is an autosomal recessive disease caused by impaired steroidogenesis, and 21-hydroxylase deficiency (21OHD) accounts for approximately 95% of cases [1]. Since adults with 21OHD exhibit a broad spectrum of enzyme deficiency and clinical features, it is challenging to achieve adrenal androgen suppression and optimal steroid re-

placement simultaneously. Undertreatment results in androgen excess and adrenal insufficiency, while overtreatment causes iatrogenic Cushing syndrome and metabolic syndrome, the components of which include obesity and hypertension [2,3]. Traditionally, 17α-hydroxyprogesterone (17-OHP) has been measured as the therapeutic indicator for hormone control. In recent studies, androstenedione, 21-deoxycortisol, and 11-oxygenated androgens have been recommended as reliable markers for disease control; however, these hormone measurements are not available in clinical practice [4]. Additionally, the optimal target levels of these hormones have not been established since the reference range and sampling time vary [4]. Thus, another marker for reflecting disease control is needed.

Adrenal volumetry has been used to evaluate the dimensions of normal adrenal glands and to characterize specific diseases that may affect the adrenal parenchyma [5-9]. Adrenal imaging is not routinely recommended in patients with 21OHD [4], but may be considered in patients with a long history of poor disease control, inconsistent therapy, or difficult-to-control disease as a rule of thumb [10]. Previous studies have investigated the correlation between adrenal volume and hormonal status [11,12]. However, the clinical significance of adrenal volume in patients with 21OHD has not been well demonstrated.

We compared the adrenal morphology, including limb width and volume, between classical 21OHD patients and healthy controls. Furthermore, we aimed to gather more evidence on the relationship between adrenal volume and biochemical parameters in patients with 21OHD and to evaluate the diagnostic value of adrenal volume as a long-term disease control marker.

METHODS

Study population

The Institutional Review Board of Seoul National University Hospital approved this retrospective study and waived the requirement for informed consent (IRB no.: H-2004-147-1118). Between November 2016 and March 2021, we identified 308 patients with 21OHD who visited the endocrine clinic of our center. Among these, 95 patients fulfilled the following inclusion criteria: (1) age >18 years; (2) underwent abdominal computed tomography (CT) for adrenal imaging; and (3) underwent hormonal evaluation on the same day as the CT scan. We excluded three patients with non-classical 21OHD and two patients who underwent unilateral adrenalectomy. This study included a final total of 90 patients (44 men and 46 women; mean age, 29.7±7.9 years).

For comparisons of adrenal morphology and volume, this study included 270 age- and sex-matched controls (135 men and 135 women with a mean age of 31.7±3.9 years) who underwent abdominal CT for health screening. We excluded patients with adrenal masses or any known endocrine diseases that might affect their adrenal glands.

Clinical and biochemical data

We reviewed each patient's electronic medical records and retrieved data from the visit for CT imaging. The body mass index (BMI) was defined as body weight divided by height squared (kg/m^2). We measured blood pressure using an automated technique in a seated position after a 20-minute rest.

We also assessed each patient's current glucocorticoid and fludrocortisone regimen. The daily glucocorticoid dose equivalents were calculated as follows: hydrocortisone, 1; prednisolone/prednisone, 4; and dexamethasone, 70 [13]. Glucocorticoid dose equivalents and the fludrocortisone dose were divided by body surface area (BSA) for normalization.

Morning fasting blood samples for laboratory tests were drawn before patients took steroid medications. Laboratory tests included plasma adrenocorticotropic hormone (ACTH), 17-OHP, androstenedione, 11β-hydroxytestosterone (11β-OHT), 11β-hydroxyandrostenedione (11β-OHA), testosterone, progesterone sulfate (Preg-S), total testosterone, and plasma renin activity. We categorized 21OHD patients into well-controlled and poorly-controlled groups according to serum 17-OHP levels [4], using a 17-OHP level lower than 10 ng/mL as a cut-off for the well-controlled group corresponding to a level two-fold higher than the upper normal limit [3].

We included obesity, hyperglycemia, hypertension, and dyslipidemia as metabolic morbidities, which were modified from the harmonizing criteria of metabolic syndrome [14]. The definition of each metabolic morbidity is described in the supplementary materials.

CT protocol

All patients with 21OHD underwent either non-contrast ($n=83$) or contrast-enhanced abdominal CT ($n=7$) using multi-detector CT scanners (64 to 128 channels). In the control group, all patients underwent contrast-enhanced abdominal CT ($n=270$). CT scans were performed in the supine position with a scan range from the top of the diaphragm to the inferior margin of the symphysis pubis. All scans were acquired in a single breath-hold to minimize motion and misregistration artifacts. The detailed settings were as follows: collimation, 64×0.625 mm, or 128×0.6

mm; gantry rotation time, 0.5 second; pitch, 0.891 or 0.65; slice thickness, 3 mm; reconstruction interval, 3 mm; and matrix, 512×512.

Images were acquired using 80 to 120 kVp tube energy and automatic tube current modulation technology available for each vendor (Care Dose 4D, Siemens Medical Solutions, Erlangen, Germany; Dose Right and Tube Current Modulation, Philips Medical Systems, Best, The Netherlands; or AutomA, GE Medical Systems, Milwaukee, WI, USA). For contrast-enhanced CT, portal phase images were obtained 70 seconds after administering the contrast medium. Iodinated contrast medium at a concentration of 350 mgI/mL was administered into the peripheral vein of the upper extremity via an automatic power injector at a total dosage of 1.5 mL/kg over 30 seconds.

Imaging analysis

Two radiologists (T.M.K. and S.Y.K. with 6 and 16 years of genitourinary imaging experience, respectively) who were blinded to the related clinical information reviewed all CT images using a picture archiving and communication system (Infinitt, Infinitt Healthcare, Seoul, Korea).

Adrenal morphology was classified into hypertrophy, normal size, and hypotrophy according to a limb width ≥5 mm, ≥2 mm and <5 mm, and <2 mm, respectively [8]. We measured the widths of the adrenal limbs at the widest part of the medial or lateral limbs perpendicular to the long axis of the limb in both adrenal glands and calculated the average of those measurements (Fig. 1). The readers carefully examined whether adrenal masses were present. A diagnosis of myelolipoma was considered when the readers detected gross fat inside the mass; other-
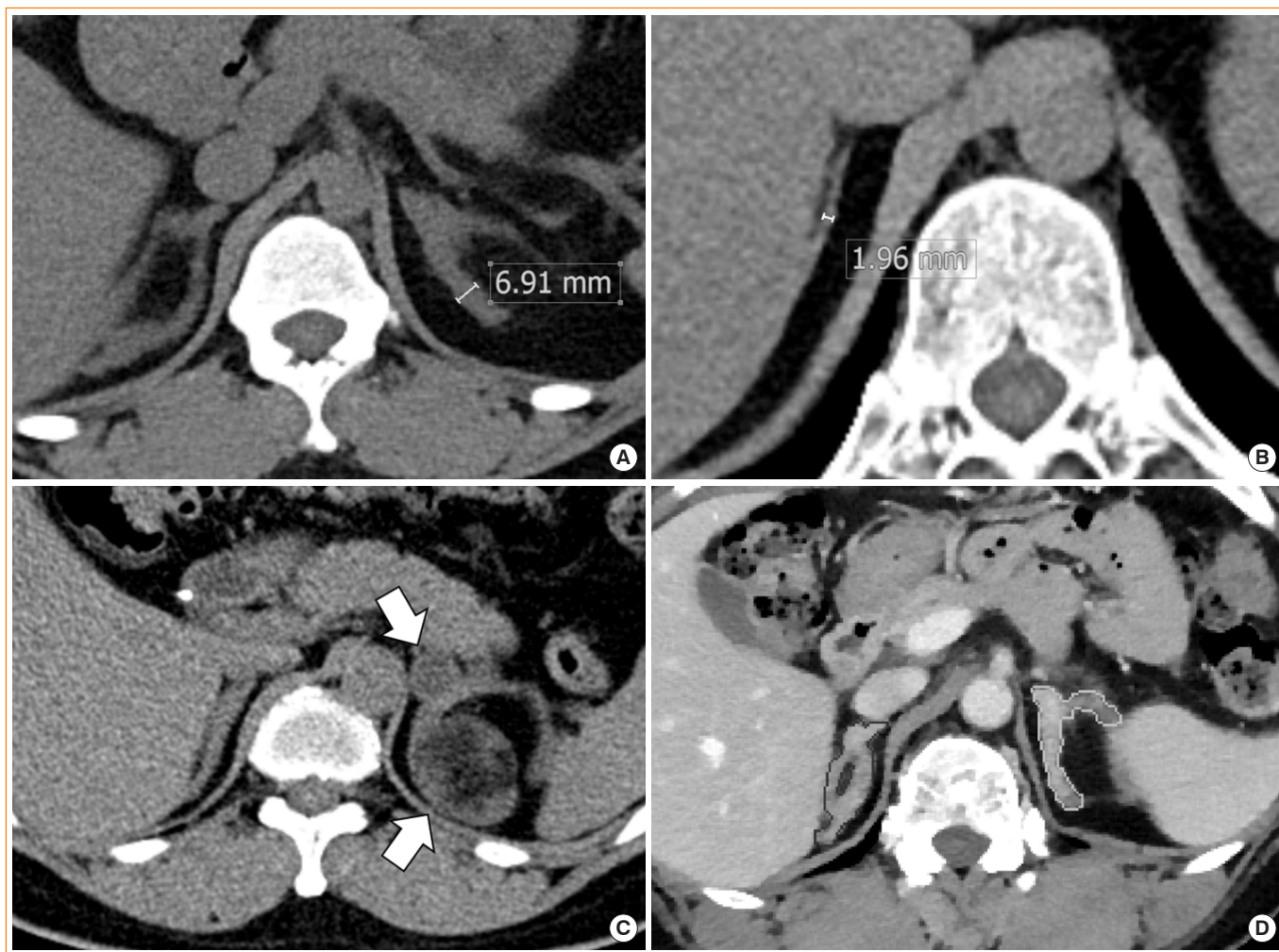
Fig. 1. Image analysis of patients with 21-hydroxylase deficiency (21OHD). We evaluated the adrenal morphology according to (A, B) limb width, (C) the presence of an adrenal mass (arrows), and (D) adrenal volume by manual segmentation.

wise, it was considered an adenoma. In the case of a disagreement, a third radiologist (J.Y.C., 26 years of genitourinary imaging experience) who was also blinded to the patients' clinical information provided input.

For volumetric evaluation of the adrenal glands, one radiologist (T.M.K.) manually segmented the adrenal parenchyma on each side in axial CT images using commercially available software (MEDIP, Medical IP, Seoul, Korea). To make volumetric measurements, we manually drew the margin of the adrenal glands as close as possible to the surface of the gland to exclude adjacent fatty tissue, the liver, the inferior vena cava, spleen, and the pancreatic tail. We carefully avoided the tumor because we wanted to focus on the parenchymal volume. The total adrenal volume was computed by combining both sides of the adrenal parenchymal volume. We also segmented the adrenal tumors independently of the parenchyma, and the total tumor volume was calculated as the sum of all segmented tumors. All processes were performed in a standard abdominal window setting with a width of 300 and a level of 40. To evaluate interobserver reliability, another radiologist (S.Y.K.) performed additional measurements of 90 21OHD patients in the same manner.

Additionally, we performed whole-body composition analysis using a dedicated software program (DEEPCATCH, Medical IP). The software program provides fully automatic segmentation of body components with an average segmentation accuracy of 97% compared with manual segmentation [15]. The L3 sectional area (cm^2) of skeletal muscle, abdominal visceral fat, and subcutaneous fat were automatically quantified. The total abdominal fat area was calculated by summing the abdominal visceral fat and subcutaneous fat areas. In addition, the waist circumference (cm) was automatically measured in the level of the upper tip of the hip bone. One endocrinologist (H.N.J.) confirmed the results of automatic segmentation. We compared the body composition of the 21OHD patients between the well-controlled and poorly-controlled groups.

Biochemical assays

Plasma ACTH levels were measured using an immunoradiometric assay (Cisbio Bioassays, Saclay, France) with intra- and inter-assay coefficients of variation (CVs) of 3.7% and 3.8%. Radioimmunoassay (RIA) CT kits (Asbach Medical Products GmbH, Obrigheim, Germany) were used to measure serum 17-OHP and dehydroepiandrosterone sulfate (DHEA-S) levels. The intra- and inter-assay CVs for 17-OHP levels were 4.6% and 7.7%, respectively, and the respective CVs for DHEA-S levels were 3.6% and 6.5%. Serum total testosterone levels

were measured with a TESTO-CT2 kit (Cisbio Bioassays). The intra- and inter-assay CVs for total testosterone were 3.1% and 5.2%, respectively. Plasma renin activity was gauged with a PRA RIA kit (TFB Inc., White Lake, MI, USA), with intra- and inter-assay CVs of 3.8% and 6.7%, respectively.

Adrenal steroids, including androstenedione, 11β-OHT, 11β-OHA, testosterone, and Preg-S were quantitatively profiled by liquid chromatography-mass spectrometry (LC-MS), as described previously [16]. Based on individual serum concentrations, we also calculated the metabolic ratio of 11β-hydroxy-testosterone to testosterone (11β-OHT/T) to indicate testicular 11β-hydroxylase activity.

Definition of metabolic morbidity

Patients with a BMI >25 kg/m^2 were considered obese. Hyperglycemia, which included diabetes mellitus and prediabetes, was defined as a hemoglobin A1c value ≥5.7%, a fasting plasma glucose level ≥100 mg/dL, or the use of any oral anti-diabetic drugs or insulin therapy. Hypertension was defined as a systolic blood pressure ≥130 mm Hg and/or diastolic blood pressure ≥85 mm Hg or the use of any antihypertensive medications. Dyslipidemia was defined as an abnormal lipid panel (total cholesterol ≥240 mg/dL, low-density lipoprotein cholesterol ≥160 mg/dL, triglycerides ≥150 mg/dL, or high-density lipoprotein cholesterol <40 mg/dL) or the use of lipid-lowering agents.

Statistical analysis

For group comparisons, we used the Fisher exact test for categorical variables and the independent t test or the Mann–Whitney U test for continuous variables, as appropriate. The Kruskal–Wallis test with Conover *post hoc* analysis was performed to compare 17-OHP and androstenedione among the 21OHD patients according to adrenal morphology. We conducted subgroup analyses of the total adrenal volume according to the phenotype, regularity of menstruation, and presence of adrenal tumors. We used Spearman correlation coefficients for correlation analysis, for which non-normally distributed variables (all biochemical parameters and adrenal volume) were log-transformed.

A receiver operating characteristic analysis was performed to evaluate the diagnostic accuracy of adrenal volume and width for discriminating 21OHD patients among the normal population, as well as to predict the well-controlled group and patients with at least one metabolic morbidity. Interobserver agreement was evaluated using the intraclass correlation coefficient (ICC), with ICC values of <0.4, 0.4–0.6, 0.6–0.8, and >0.8 interpreted

as indicating poor, moderate, good, and excellent agreement, respectively. All statistical analyses were performed using SPSS version 25 (IBM, Armonk, NY, USA), and *P* values of <0.05 were considered statistically significant.

RESULTS

Patient characteristics
Detailed information on the study group is summarized in Table

1. Forty-four (48.9%) of the 90 patients with 21OHD were men. Forty-six patients (51.1%) had the salt-wasting type of 21OHD, while 44 patients (48.9%) had the simple virilizing type. There were no significant differences in the biochemical parameters and glucocorticoid doses between the salt-wasting and simple virilizing types (Table 2). All 90 patients were treated with prednisolone, two patients with hydrocortisone, and 55 with fludrocortisone. The median values of daily glucocorticoid dose/BSA and fludrocortisone dose/BSA were 17.5 mg/m^2 (range, 5.2 to

Table 1. Clinical Characteristics of the Patients

Characteristic	Total (*n*=90)	Male (*n*=44)	Female (*n*=46)	*P* value[a]
Age, yr	28 (19–55)	28 (19–55)	28.5 (19–54)	0.42
Height, cm	158.1 (146.8–182.3)	164.7 (146.8–182.3)	152.6 (133–166.4)	<0.01
Weight, kg	63.5 (53.9–103.1)	69.3 (53.9–103.1)	59.9 (42–93.4)	<0.01
BMI, kg/m^2	25.0 (19.7–41.3)	25.2 (19.7–38.5)	24.6 (18–41.3)	0.47
Phenotype				0.28
Salt-wasting	46 (51.1)	20 (45.5)	26 (56.5)	
Simple virilizing	44 (48.9)	24 (54.5)	20 (43.5)	
Metabolic risk factors	56 (62.2)	33 (75)	22 (47.8)	0.01
Obesity	35 (38.9)	20 (45.5)	15 (32.6)	0.21
Hyperglycemia	25 (27.8)	13 (29.5)	12 (26.1)	0.39
Hypertension	17 (18.9)	11 (25)	6 (13.0)	0.15
Dyslipidemia	21 (23.3)	12 (27.3)	9 (19.6)	0.39
Glucocorticoid type				
Hydrocortisone	2 (2.2)	1 (2.3)	1 (2.2)	0.98
Prednisolone	90 (100)	44 (100)	46 (100)	NA
Glucocorticoid-dose/BSA, mg/m^2/day	17.5 (5.2–26.7)	16.5 (5.2–26.7)	18.9 (7.4–24.5)	0.02
Fludrocortisone use	55 (61.1)	28 (63.6)	27 (58.7)	0.63
ACTH[b], pg/mL	96.9 (2.7–5,030)	107.4 (3.3–5,030)	58.9 (2.7–726)	0.04
17-OHP, ng/mL	34.3 (0.6–344)	36.2 (1.6–126)	32.6 (0.6–344)	0.75
17-OHP <10 ng/mL	26 (28.9)	11 (25)	15 (32.6)	0.43
Androstenedione[b], ng/mL	1.3 (0.1–11.3)	1.5 (0.2–9.8)	1.2 (0.1–11.3)	0.58
11β-OHT[b], ng/mL	0.4 (0.02–15.1)	0.6 (0.02–15.1)	0.4 (0.03–4.6)	0.20
11β-OHA[b], ng/mL	3.2 (0.02–51.8)	6.4 (0.02–51.8)	3.0 (0.02–44.4)	0.21
Preg-S[b], ng/mL	95.5 (2.5–1,201.2)	133.6 (12.8–1,045.3)	70.7 (2.5–1201.2)	0.20
DHEA-S[b], ng/mL	632 (101–5,169)	1,097 (248.2–5,017)	403 (101–5,169)	0.17
Testosterone[b], ng/mL	1.3 (0.08–7.3)	4.8 (2.0–7.3)	0.4 (0.08–3.7)	<0.01
Androstenedione/testosterone[b]	2.0 (0.05–6.3)	2.8 (0.9–6.3)	0.3 (0.05–2.6)	<0.01
Plasma renin activity[b], ng/mL	7.2 (0.1–75.2)	6.9 (0.1–75.2)	7.1 (1.4–49.5)	0.60

Values are expressed as median (range) or number (%).
BMI, body mass index; NA, not applicable; BSA, body surface area; ACTH, adrenocorticotropic hormone; 17-OHP, 17α-hydroxyprogesterone; 11β-OHT, 11β-hydroxytestosterone; 11β-OHA, 11β-hydroxyandrostenedione; Preg-S, progesterone sulfate; DHEA-S, dehydroepiandrosterone sulfate.
[a]Continuous data were assessed using the Mann–Whitney *U* test, while categorical data were assessed using Fisher's exact test; [b]Data were not available for some of the patients (ACTH, *n*=2; androstenedione, 11β-OHT, 11β-OHA, 11β-hydroxytestosterone/testosterone [11β-OHT/T], Preg-S, testosterone, *n*=44; DHEA-S, *n*=59; plasma renin activity, *n*=6).

26.7) and 0.06 mg/m² (range, 0.03 to 0.40), respectively. The ACTH level and androstenedione/T ratio were significantly higher in male than in female patients ($P=0.02$ and $P<0.001$, respectively), while 11β-OHT/T was significantly higher in female than in male patients ($P<0.001$). Fifty-six out of 90 patients (62.2%) had at least one metabolic morbidity, and the male patients had a significantly higher incidence of metabolic morbidity than female patients (75% vs. 47.8%, $P=0.01$). The biochemically well-controlled group comprised 26 patients (28.9%, 11 men and 15 women), while the poorly-controlled group contained the remaining 64 patients.

Radiologic findings of classical 21OHD patients
Among the 90 patients, 41 (45.6%) had adrenal hypertrophy, 38 (42.2%) had a normal appearance, and 11 (12.2%) had adrenal hypotrophy. In the well-controlled group, one patient showed hypertrophy, while 15 and 10 patients had a normal appearance and hypotrophy of the adrenal glands, respectively. In the poorly-controlled group, 40, 23, and 1 patients showed hypertrophy, normal appearance, and hypotrophy of the adrenal glands, respectively. Twelve patients (13.3%) had adrenal tumors, which were unilateral in nine patients and bilateral in three patients. Among them, 11 patients showed gross fat in CT images, confirming the presence of myelolipoma. The other patient present-

ed with adrenal adenoma. Detailed information on the radiologic findings of 21OHD patients is summarized in Table 3.

Volumetric and linear evaluation of adrenal glands in 21OHD patients and comparison with healthy controls
The mean and standard deviation of the right, left, and total adrenal volumes of 21OHD patients were 8.5±5.5, 9.8±7.3, and

Table 3. Radiologic Findings of the Adrenal Gland in Patients with 21OHD ($n=90$)

Characteristic	No. (%)
Adrenal morphology	
Normal	38 (42.2)
Hypertrophy	41 (45.6)
Hypotrophy	11 (12.2)
Mass	12 (13.3)
Laterality	
Unilateral	9 (10)
Bilateral	3 (3.3)
Radiologic diagnosis	
Myelolipoma	11 (12.2)
Adenoma	1 (1.1)

21OHD, 21-hydroxylase deficiency.

Table 2. Comparison of Patients with Simple Virilizing Type and Salt-Wasting Type

Characteristic	Simple virilizing type ($n=44$)	Salt-wasting type ($n=46$)	P value[a]
Glucocorticoid-dose/BSA, mg/m²/day	17.9 (6.7–24.4)	17.3 (5.2–26.7)	0.65
Fludrocortisone use	22 (50)	34 (73.9)	0.02
ACTH[b], pg/mL	90.0 (3.7–5,030)	97.8 (2.7–2,299)	0.80
17-OHP, ng/mL	38.7 (0.74–344)	34.8 (0.6–344)	0.13
17-OHP <10 ng/mL	10 (22.7)	16 (34.8)	0.21
Androstenedione[b], ng/mL	1.3 (0.2–9.8)	1.2 (0.1–11.3)	0.86
11β-OHT[b], ng/mL	0.4 (0.04–7.4)	0.02 (0.5–15.1)	0.75
11β-OHA[b], ng/mL	3.0 (0.6–43.6)	0.02 (4.8–51.8)	0.88
Preg-S[b], ng/mL	98.5 (8.2–946.1)	92.4 (2.5–1,201.2)	0.99
DHEA-S[b], ng/mL	811 (101–5,169)	675.5 (168–4,407)	0.77
Testosterone[b], ng/mL	0.8 (0.2–5.4)	1.9 (0.08–7.3)	0.93
Plasma renin activity[b], ng/mL	6.0 (1.4–29.9)	8.0 (0.1–75.2)	0.18

Values are expressed as median (range) or number (%).
BSA, body surface area; ACTH, adrenocorticotropic hormone; 17-OHP, 17α-hydroxyprogesterone; 11β-OHT, 11β-hydroxytestosterone; 11β-OHA, 11β-hydroxyandrostenedione; Preg-S, progesterone sulfate; DHEA-S, dehydroepiandrosterone sulfate.
[a]Continuous data were assessed using the Mann–Whitney U test, while categorical data were assessed using Fisher's exact test; [b]Data were not available for some of the patients (ACTH, $n=2$; androstenedione, 11β-OHT, 11β-OHA, 11β-hydroxytestosterone/testosterone [11β-OHT/T], Preg-S, testosterone, $n=44$; DHEA-S, $n=59$; plasma renin activity, $n=6$).

Table 4. Linear and Volumetric Parameters between Patients with 21OHD and Patients in the Control Group

Characteristic	21OHD patients (n=90)				Control group (n=270)				P value[b]
	Total	Men (n=44)	Women (n=46)	P value[a]	Total	Men (n=135)	Women (n=135)	P value[a]	
Limb width, mm									
Right adrenal gland	4.7±1.8	4.9±1.9	4.6±1.8	0.47	3.3±0.5	3.5±0.4	3.1±0.4	<0.001	<0.001
Left adrenal gland	4.6±2.0	4.8±2.1	4.5±1.8	0.39	3.3±0.5	3.5±0.5	3.2±0.5	<0.001	<0.001
Average of both adrenal glands	4.7±1.9	4.8±2.0	4.5±1.8	0.41	3.3±0.5	3.5±0.5	3.1±0.5	<0.001	<0.001
Volume, mL									
Right adrenal gland	8.5±5.6	8.7±5.5	8.3±5.7	0.80	3.5±1.0	4.1±0.9	3.0±0.8	<0.001	<0.001
Left adrenal gland	9.9±7.4	10.8±8.4	9.0±6.3	0.26	3.6±1.1	4.3±1.0	2.9±0.6	<0.001	<0.001
Both adrenal glands	18.4±12.4	19.4±13.1	17.4±11.7	0.43	7.1±2.0	8.4±1.9	5.9±1.4	<0.001	<0.001
Mass volume, mL	67.4±81.1	84.6±100.4	46.8±53.5	0.47					

Values are expressed as mean±standard deviations.
21OHD, 21-hydroxylase deficiency.
[a]Data were compared between the men and women using the Mann–Whitney U test; [b]Data were compared between the patients with 21OHD and the control group.

18.2±12.2 mL, respectively. These values were all significantly larger than in healthy controls (3.5±1.0, 3.6±1.1, 7.1±2.0 mL, $P<0.001$ for all). The right, left, and average adrenal width were also significantly higher in 21OHD patients than in normal controls (4.7±1.8 mm vs. 3.3±0.5 mm, 4.6±2.0 mm vs. 3.3±0.5 mm, 4.7±1.9 mm vs. 3.3±0.5 mm, $P<0.001$ for all). All limb width and volume parameters were significantly higher in men than in women among the healthy controls ($P<0.001$ for all); however, there was no statistically significant difference between men and women with 21OHD ($P>0.05$ for all). We obtained an area under the curve (AUC) of 0.85 for classifying patients with 21OHD ($P<0.001$) with a sensitivity of 81.1% and specificity of 90% at a cut-off value of 9.1 mL. Table 4 presents detailed information on linear and volumetric parameters.

Subgroup analysis of adrenal volume in 21OHD patients
The adrenal volume between patients with salt-wasting 21OHD and those with the simple virilizing type was not significantly different (17.9±13.9 mL vs. 18.4±8.9 mL, $P=0.31$). Patients with irregular menstruation showed significantly larger adrenal volumes than those with regular menstruation (21.0±12.9 mL vs. 12.7±8.5 mL, $P=0.009$). In addition, patients with adrenal tumors had significantly larger adrenal volumes than those without any masses (31.8±16.1 mL vs. 15.7±9.5 mL, $P=0.025$).

Correlation analysis of adrenal volume and width with hormonal status
Both of the plasma levels of 17-OHP and androstenedione were

significantly different among groups according to adrenal morphology (hypertrophy, normal appearance, and hypotrophy; $P<0.001$ and $P<0.02$, respectively). Patients with adrenal hypertrophy showed the highest 17-OHP and androstenedione levels (median, 49.9 ng/mL [interquartile range (IQR), 22.2 to 108.7] and 2.6 ng/mL [IQR, 1.0 to 5.0], respectively), followed by those with normal adrenal glands (32.1 ng/mL [IQR, 5.1 to 58.3] and 1.1 ng/mL [IQR, 0.5 to 1.6]) and those with adrenal hypotrophy (3.6 ng/mL [IQR, 2.8 to 8.9] and 0.4 ng/mL [IQR, 0.2 to 1.1]) (Fig. 2).

The total adrenal volume correlated positively with ACTH, 17-OHP, DHEA-S, androstenedione, 11β-OHT, 11β-OHA, and Preg-S in both male and female patients ($r=0.43–0.94$, $P<0.05$ for all). Positive correlations were found for 11β-OHT/T and plasma renin activity in male patients ($r=0.49–0.70$, $P<0.05$ for both) and for testosterone in female patients ($r=0.70$, $P<0.001$) (Fig. 3). Adrenal width correlated positively with ACTH, 17-OHP, 11β-OHT, 11β-OHT/T, Preg-S, DHEA-S in both male and female patients ($r=0.31–0.95$, $P<0.05$ for all). The adrenal width showed positive correlations with androstenedione, 11β-OHA, plasma renin activity only in male ($r=0.43–0.81$, $P<0.05$ for all), and showed positive correlation with testosterone only in female ($r=0.44$, $P=0.03$). Table 5 presents detailed information on the correlation analysis.

The daily glucocorticoid dose/BSA correlated negatively with adrenal volume ($r=−0.24$, $P=0.02$), but showed no significant correlation with adrenal width ($r=−0.14$, $P=0.19$). The daily fludrocortisone dose/BSA did not show significant correlations
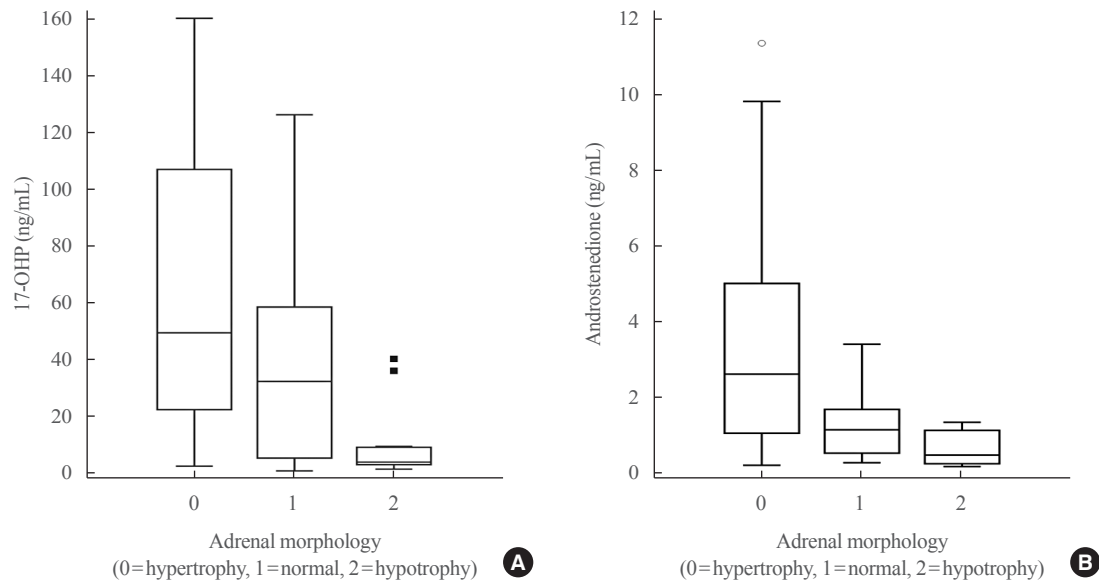
Fig. 2. Plasma levels of (A) 17α-hydroxyprogesterone (17-OHP) and (B) androstenedione according to the adrenal morphology. Patients with adrenal hypertrophy showed the highest 17-OHP and androstenedione levels, followed by those with normal adrenal glands and adrenal hypotrophy.

Table 5. Correlations of Adrenal Volume and Width with Hormonal Status in Patients with 21OHD

| Variable | Adrenal volume | | | | | | Adrenal width | | | | | |
| | Men | | | Women | | | Men | | | Women | | |
	No.	r	P value[a]	No.	r	P value[a]	No.	r	P value[a]	No.	r	P value[a]
ACTH	43	0.523	<0.001	45	0.434	0.003	43	0.326	0.03	45	0.407	0.006
17-OHP	44	0.644	<0.001	46	0.578	<0.001	44	0.627	<0.001	46	0.460	0.001
Androstenedione	20	0.592	0.006	26	0.610	<0.001	20	0.654	0.002	26	0.319	0.112
11β-OHT	20	0.681	0.001	26	0.643	<0.001	20	0.827	<0.001	26	0.558	0.003
11β-OHA	20	0.711	<0.001	26	0.658	<0.001	20	0.807	<0.001	26	0.303	0.133
11β-OHT/T	20	0.700	<0.001	26	0.181	0.377	20	0.836	<0.001	26	0.454	0.020
Preg-S	20	0.637	0.003	26	0.606	0.001	20	0.671	0.001	26	0.391	0.048
DHEA-S	10	0.941	<0.001	22	0.557	0.007	10	0.953	<0.001	22	0.617	0.002
Testosterone	20	−0.230	0.329	26	0.696	<0.001	20	−0.209	0.376	26	0.438	0.025
Plasma renin activity	39	0.488	0.002	45	0.126	0.409	39	0.431	0.006	45	0.102	0.507

21OHD, 21-hydroxylase deficiency; ACTH, adrenocorticotropic hormone; 17-OHP, 17α-hydroxyprogesterone; 11β-OHT, 11 beta-hydroxytestosterone; 11β-OHA, 11β-hydroxyandrostenedione; 11β-OHT/T, 11β-hydroxytestosterone/testosterone; Preg-S, progesterone sulfate; DHEA-S, dehydroepiandrosterone sulfate.
[a]Correlations were evaluated using the Spearman correlation coefficient. Non-normally distributed variables (adrenal volume and all biochemical parameters) were log-transformed.

with adrenal volume or width (*P*>0.05 in both).

Diagnostic performance of adrenal volume and width for the identifying 21OHD patients with well-controlled status and metabolic morbidity

The total adrenal volume and width yielded AUCs of 0.87 (95%

confidence interval [CI], 0.78 to 0.93) and 0.82 (95% CI, 0.72 to 0.89), respectively, for predicting the biochemically well-controlled group (*P*<0.001 for both) (Fig. 4). We could achieve a sensitivity of 73.1% and specificity of 90.6% using a cut-off value of 10.7 mL of adrenal volume. Using a cutoff value of 4.0 mm of adrenal width, a sensitivity of 80.8% and specificity of
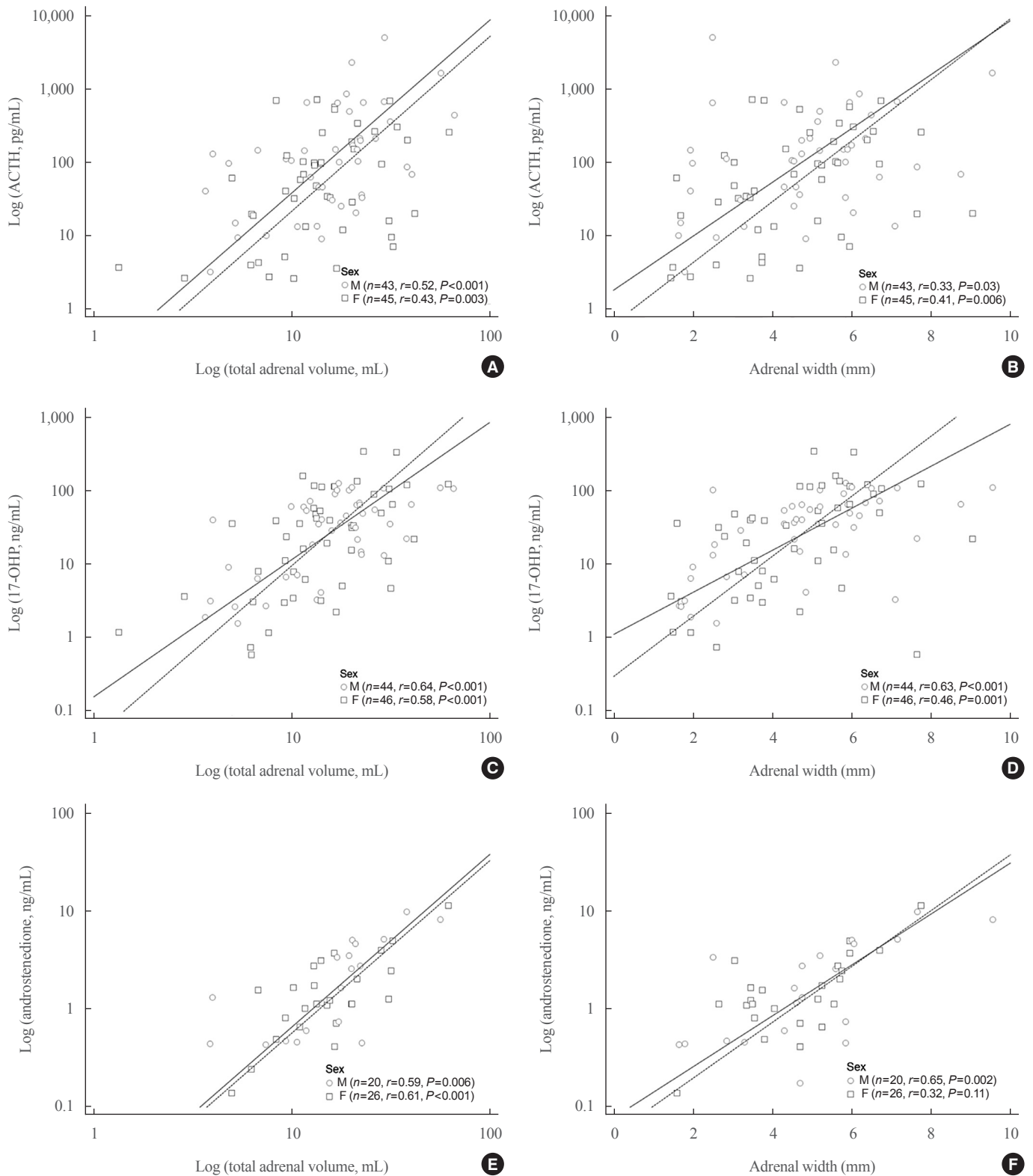
Fig. 3. Correlations between total adrenal volume and hormonal status and adrenal width and hormonal status. Both total adrenal volume and adrenal width correlated positively with (A, B) adrenocorticotropic hormone (ACTH) and (C, D) 17α-hydroxyprogesterone (17-OHP) levels regardless of sex. (E, F) Androstenedione levels correlated positively with adrenal volume in both male and female patients, and with adrenal width in male patients.
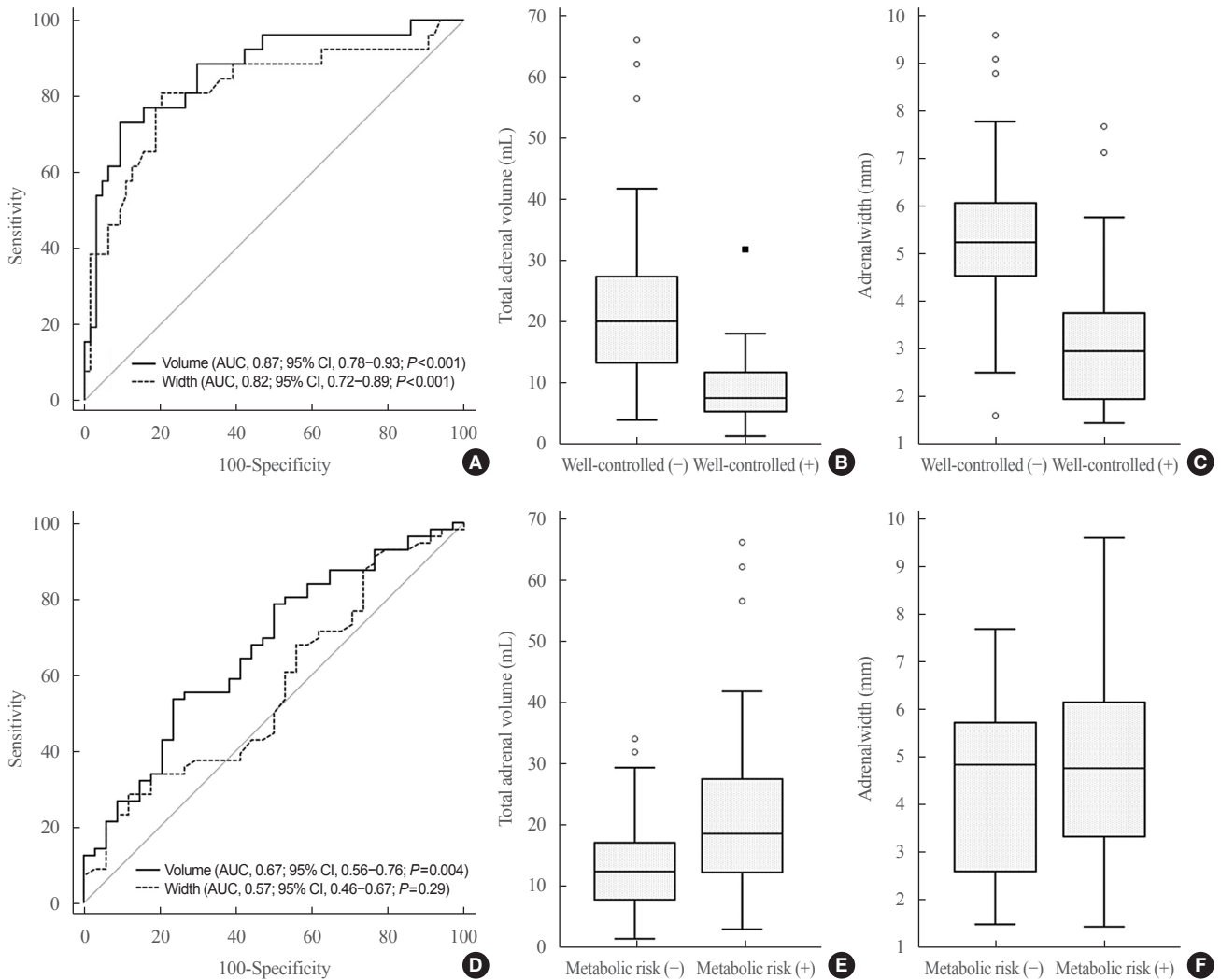
Fig. 4. Receiver operating characteristic curves and box-and-whisker diagrams of adrenal volume and width for classifying well-controlled patients (A, B, C) and patients with at least one metabolic morbidity (D, E, F), respectively. AUC, area under the curve; CI, confidence interval.

79.7% were obtained. The AUCs of adrenal volume and width showed no significant differences ($P=0.24$).

The total adrenal volume resulted in an AUC of 0.67 (95% CI, 0.56 to 0.76) for predicting patients with one or more metabolic morbidity ($P=0.004$). Using a cut-off value of 17 mL, a sensitivity of 55.4% and specificity of 76.5% were obtained. Adrenal width showed no significant diagnostic value for predicting the patients with a metabolic morbidity (AUC, 0.57; 95% CI, 0.46 to 0.67; $P=0.29$). The AUC of adrenal volume was significantly higher than that of adrenal width ($P=0.03$).

Correlation analysis between hormonal status and adrenal tumors

Patients with adrenal tumors showed significantly higher ACTH,

11β-OHA, and Preg-S levels than those without adrenal tumors ($P<0.05$ for all). Table 6 presents detailed information. The total volume of adrenal tumors did not show a significant correlation with any hormone levels ($P>0.05$ for all).

Comparison of body composition between well-controlled and poorly-controlled patients

The BMI was significantly higher in the poorly-controlled group than in the well-controlled group ($P=0.006$). The poorly-controlled group showed a significantly higher area of subcutaneous fat and total abdominal fat in the L3 level than the well-controlled group ($P=0.04$ in both). The visceral fat and muscle areas did not differ significantly between the two groups ($P>0.05$). The waist circumference was significantly higher in

Table 6. Comparisons of Hormonal Status between Patients with and without Adrenal Tumors

Biochemical parameters[a]	Adrenal tumor (+) (n=12)	Adrenal tumor (–) (n=78)	P value[b]
ACTH	259 (100.5–686.0)	87.7 (18.6–195.7)	0.02
17-OHP	49.2 (25.2–95.5)	31.6 (6.4–65.0)	0.25
Androstenedione	3.3 (1.7–7.6)	1.2 (0.5–2.8)	0.15
11β-OHT	2.3 (1.0–3.8)	0.4 (1.5–0.9)	0.07
11β-OHA	23.8 (12.9–34.4)	3.0 (0.7–7.3)	0.03
11β-OHT/T	1.4 (1.0–1.5)	0.7 (0.1–1.0)	0.12
Preg-S	807.3 (399–1,052)	82.2 (31–246)	0.03
DHEA-S	632	675.5 (346–1,721)	0.92
Testosterone	2.1 (1.1–3.0)	1.1 (0.3–4.8)	0.87
Plasma renin activity	11.0 (7.7–14.8)	6.9 (4.3–12.5)	0.14

Values are expressed as median (range).
ACTH, adrenocorticotropic hormone; 17-OHP, 17α-hydroxyprogesterone; 11β-OHT, 11β-hydroxytestosterone; 11β-OHA, 11β-hydroxyandrostenedione; 11β-OHT/T, 11β-hydroxyandrostenedione/testosterone; Preg-S, progesterone sulfate; DHEA-S, dehydroepiandrosterone sulfate.
[a]Data were not available for some of the patients (ACTH, n=2; androstenedione, 11β-OHT, 11β-OHA, 11β-OHT/T, Preg-S, testosterone, n=44; DHEA-S, n=59; plasma renin activity, n=6); [b]Continuous data were compared using the Mann–Whitney U test.

Table 7. Comparisons of Body Composition, Adrenal Morphology, and Biochemical Parameters between Well-Controlled and Poorly-Controlled Patients

Parameter	Well-controlled (n=26)	Poorly-controlled (n=64)	P value[a]
BMI, kg/m^2	24.3±4.7	26.9±4.9	<0.01
Subcutaneous fat area, cm^2	163.7±74.7	202.3±81.9	0.04
Visceral fat area, cm^2	88.1±47.3	110.9±63.3	0.10
Total abdominal fat area, cm^2	251.8±113.5	313.2±131.1	0.04
Muscle area, cm^2	116.0±23.4	126.3±31.5	0.14
Waist circumference, cm	86.9±11.0	94.8±15.2	0.02
Adrenal volume, mL	9.4±6.2	21.6±12.3	<0.01
Adrenal width, mm	3.2±1.7	5.2±1.6	<0.01
ACTH[b]	11.3 (2.7–148.8)	139.6 (7.4–5,030)	<0.01
17-OHP	3.3 (0.6–9.2)	51.6 (11.1–344)	<0.01
Androstenedione[b]	0.7 (0.2–3.1)	1.3 (0.1–11.3)	0.06
11β-OHT[b]	0.09 (0.02–0.8)	0.7 (0.02–15.1)	<0.01
11β-OHA[b]	0.6 (0.02–5.5)	5.0 (0.02–51.8)	<0.01
11β-OHT/T[b]	0.1 (0.003–1.5)	0.8 (0.007–2.8)	0.02
Preg-S[b]	43.8 (12.8–320.8)	149.2 (2.5–1,201)	0.04
DHEA-S[b]	387 (101–2,016)	1,328 (228–5,169)	0.04
Testosterone[b]	1.0 (0.1–6.8)	1.9 (0.08–7.3)	0.98
Plasma renin activity[b]	6.6 (0.1–29.9)	7.8 (1.4–75.2)	0.45

Values are expressed as mean±standard deviations or median (range).
BMI, body mass index; ACTH, adrenocorticotropic hormone; 17-OHP, 17α-hydroxyprogesterone; 11β-OHT, 11β-hydroxytestosterone; 11β-OHA, 11β-hydroxyandrostenedione; 11β-OHT/T, 11β-hydroxyandrostenedione/testosterone; Preg-S, progesterone sulfate; DHEA-S, dehydroepiandrosterone sulfate.
[a]Data were compared using the independent t test for normally distributed variables (all of computed tomography parameters) or the Mann–Whitney U test for non-normally distributed variables (body mass index, adrenal volume, adrenal width, all of biochemical parameters); [b]Data were not available for some of the patients (ACTH, n=2; androstenedione, 11β-OHT, 11β-OHA, 11β-OHT/T, Preg-S, testosterone, n=44; DHEA-S, n=59; plasma renin activity, n=6).

the poorly-controlled group than in the well-controlled group (*P*=0.02). Table 7 summarizes detailed information on body composition.

Interobserver agreement
The interobserver agreement for adrenal width measurements was 0.689, 0.598, and 0.685 (*P*<0.001 for all) for the right, left, and average adrenal widths, respectively. All adrenal volume parameters, including the right, left, and both adrenal glands, showed excellent agreement (ICC, 0.874, 0.906, 0.888, respectively; *P*<0.001 for all).

DISCUSSION

Our study demonstrated that the total adrenal volume and adrenal width measured by abdominal CT reflected the extent of disease control in patients with 21OHD. Thus, adrenal volume and width can be useful for identifying 21OHD patients with well-controlled status or at least one metabolic morbidity. To our knowledge, this is the first study to predict the disease control status of 21OHD patients using adrenal volume and width with optimal cut-off values.

Reisch et al. [12] first evaluated the association between adrenal volume and hormonal control in patients with 21OHD. They found that adrenal volume correlated positively with androstenedione, 17-OHP in serum, and pregnanetriol in urine. However, the number of patients included in the study was small (*n*=26), and all of them were male. This might be the reason why ACTH, plasma renin activity, and DHEA-S did not show significant correlations with adrenal volume in their study, unlike the findings of the current study. Adina et al. analyzed correlations of a mass-spectrometry panel of 23 steroids with disease control status, including adrenal volume, and found that 11-oxygenated androgens could serve as clinically useful biomarkers [11]. However, these hormone measurements are not currently available in clinical practice, limiting their clinical usefulness. Above all, those two studies did not suggest a specific cut-off value of adrenal volume for evaluating the disease control status. This study is the first to set cut-off volumes to classify well-controlled patients and patients with at least one metabolic morbidity (10.7 and 17 mL, respectively), with acceptable diagnostic accuracy (AUC, 0.87 and 0.67, respectively).

A remarkable finding of our study is that we found the clinical significance of adrenal gland hypotrophy in patients with 21OHD. Secondary adrenal hypoplasia is known to result from ACTH suppression. Surprisingly, 11 patients (12.2%) showed diffuse hypotrophy of the adrenal gland, in contrast to the typical adrenal morphology of patients with 21OHD. The 17-OHP and androstenedione levels were lower in patients with adrenal hypotrophy than in those with normal adrenal glands and adrenal hypertrophy. Of particular note, half of the patients with adrenal hypotrophy showed normal values of 17-OHP, which suggests overtreatment [4]. One patient showed adrenal hypotrophy, but had a high 17-OHP level (33.1 ng/mL). Because the adrenal morphology is thought to be affected by long-term hormonal status, it would not reflect very recent hormonal changes. Therefore, the adrenal morphology may not perfectly represent the hormonal state at the time of the CT scan. Nevertheless, when we detect adrenal gland hypotrophy in patients with 21OHD, we should beware of overtreatment.

Accurate volumetric measurement of the adrenal gland is crucial because of its high interobserver variability owing to its small volume [17]. CT is considered the gold standard for volume measurement of organs *in vivo*. Magnetic resonance imaging (MRI) can also produce high-resolution volume data; however, magnetic field inhomogeneity combined with chemical shift artifacts can result in spatial inhomogeneity. We used multi-detector CT with thin slices of 3 mm to measure the adrenal volume. Our data are expected to be more accurate than those of other studies that measured the adrenal volume on CT with thick slices of ≥5 mm or those conducted with MRI [8,12,18].

We performed both volumetric and linear assessments of the adrenal gland to compare the diagnostic performance. We hypothesized that the complex shape of the adrenal glands could complicate the measurement of the adrenal width in two-dimensional CT images. As we expected, the reproducibility of adrenal width (good agreement, ICC 0.685) was inferior to that of adrenal volume (excellent agreement, ICC 0.888). However, adrenal width showed a good correlation with biochemical parameters. Furthermore, it showed good diagnostic performance for predicting biochemically well-controlled patients (AUC, 0.82). Adrenal width can be easily applied in real clinical practice considering its rapidity of measurement.

Our study demonstrated the prevalence of adrenal masses in adult patients with classic 21OHD who were on medication. Approximately 13.3% of the patients had adrenal tumors, which is much lower than the value reported in a previous study [19]. Moreover, only one patient (1.1%) had adenoma in our study, while most tumors were described as "silent adenomas" in a previous study [19]. This discrepancy might be attributed to differences in image interpretation. Adrenal hyperplasia is a major

condition that is often falsely diagnosed as adenoma due to a significant overlap in imaging findings [20,21]. A majority of patients with 21OHD who were considered to have adenomas in previous studies may actually have had adrenal hyperplasia. In addition, all the patients in the current study with adrenal tumors, except for one, had myelolipomas. Some patients with untreated 21OHD with very high serum ACTH levels occasionally demonstrate large myelolipomas [22,23]. Chronic ACTH stimulation might play an important role in myelolipoma development [24]. In concordance with these findings, patients with adrenal tumors showed higher ACTH levels than those without tumors in our study. This finding can be interpreted as indicating that adrenal imaging should be considered in patients with 21OHD with high ACTH levels to identify adrenal incidentalomas, especially myelolipomas.

It is well known that patients with 21OHD have a higher prevalence of metabolic morbidities than the normal population [3,25]. Adrenal volume was useful in identifying 21OHD patients with at least one metabolic morbidity (AUC, 0.67). We also found that total abdominal fat area and waist circumference were significantly different according to patients' hormonal control status.

Our study had several limitations. First, this study was performed retrospectively at a single center; thus, there may have been selection bias. Second, the hormones examined were different for each patient depending on the clinician's determination. In addition, single measurement results of the hormones were analyzed. In particular, patients with 21OHD were classified into well-controlled and poorly-controlled groups according to a single measurement of 17-OHP levels. Third, not all patients in our cohort were genetically confirmed to have classical 21OHD. However, all patients showed typical hormone abnormalities and a clinical course consistent with classical 21OHD. Finally, measuring adrenal volume is time-consuming and laborious compared with measuring adrenal width. For application in clinical practice, automatic or semi-automatic volumetric assessment of the adrenal gland is warranted.

In conclusion, adrenal volume and width were associated with hormonal status in patients with 21OHD and showed good diagnostic performance in identifying well-controlled patients and patients with at least one metabolic morbidity. Given the lifetime duration of the disease course, both adrenal volume and width are reliable quantitative parameters for monitoring patients with classical 21OHD.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: T.M.K., J.H.K., H.N.J., M.H.C., J.Y.C., S.Y.K. Acquisition, analysis, or interpretation of data: T.M.K., J.H.K., H.N.J., M.H.C., J.Y.C., S.Y.K. Drafting the work or revising: T.M.K., J.H.K., H.N.J., M.H.C., J.Y.C., S.Y.K. Final approval of the manuscript: T.M.K., J.H.K., H.N.J., M.H.C., J.Y.C., S.Y.K.

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