MEDICAL PRACTICE

Use of urinary steroid profiling for diagnosing and monitoring adrenocortical tumours

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It has been suggested that urinary steroid profiling may be used to provide information aiding the diagnosis and monitoring of adrenocortical carcinoma. Nonetheless, the abnormal patterns suggestive of adrenal malignancy are not well defined. We retrospectively studied the urinary steroid profiles of five patients with adrenocortical carcinoma at presentation and at follow-up, and compared these results with those from 76 patients with benign adrenocortical adenoma and 172 healthy controls. Three abnormal patterns of urinary steroid excretion were identified in patients with adrenocortical carcinoma at presentation and/or follow-up of residual disease: (1) hypersecretion in multiple steroid axes; (2) excretion of unusual metabolites, notably 5-pregnene-3α,16α,20α-triol, 5-pregnene-3β,16α,20α-triol, and neonatal steroid metabolites in the post-neonatal period; (3) increase of tetrahydro-11-deoxycortisol relative to total cortisol metabolites. These preliminary findings offer ways in which urinary steroid profiling performed using gas chromatography–mass spectrometry can be helpful in the diagnosis and monitoring of adrenocortical carcinoma.

Introduction

The widespread application of sophisticated imaging techniques means adrenal nodules, which have a prevalence of around 6% in the general population,[1] are being detected more often. Most are benign adrenocortical adenomas (ACA) but early recognition of the much rarer adrenocortical carcinoma (ACC) is important because its mortality rate is high when the diagnosis is delayed.[2]

The adrenal cortex produces cortisol, corticosterone, and C19 steroids. Adrenal steroids are metabolised in the liver and the kidney, then excreted in the urine either in the form of free steroids or conjugated with sulphuric or glucuronic acid. Adrenal carcinoma tissues have been reported to express enzymes in the steroidogenic pathways aberrantly, leading to the increased production of normal adrenal steroids as well as unusual steroids such as metabolites of 11-deoxycortisol (compound S), steroid hormone precursors, and neonatal steroids.[2-7] Most of these adrenal steroids and metabolites can be unselectively identified and quantified by urinary steroid profiling (USP) using gas chromatography–mass spectrometry (GC-MS). In this article, we describe the clinical and laboratory findings of five cases of ACC identified in our centre between 2003 and 2005, and compare the USP findings of these patients with those of 76 patients with benign ACA and 172 healthy controls.

Key words
Adrenocortical adenoma; Adrenocortical carcinoma; Steroids/urine

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Subjects

Patients with adrenocortical carcinoma

Patient 1

This was a 51-year-old female who was referred to us in 2003 with Cushing's syndrome (CS). She had hypertension, oligomenorrhoea, a moon face, a buffalo hump, striae over her abdomen and thighs, truncal obesity, and mild hirsutism. Investigations revealed hypokalaemia of 3.0 mmol/L. Her urinary-free cortisol (UFC) levels were 1733 and 2019 nmol/d on two separate collections (reference interval [RI]: 100-379). Her cortisol levels after being given 1 mg and 8 mg overnight dexamethasone suppression were 546 nmol/L and 545 nmol/L, respectively. Her adrenocorticotropic hormone (ACTH) level was less than 2.2 pmol/L (RI: <10.1) and her dehydroepiandrosterone sulphate (DHEAS), erect plasma renin activity (PRA), and aldosterone levels were all normal. Urinary steroid profiling detected increased excretion of total cortisol metabolites (FM), and an excess of tetrahydro-11-deoxycortisol (THS) [665 μg/d; RI: 9-59], 5-pregnene-3α,16α,20α-triol (128 μg/d; RI: 5-44), and 5-pregnene-3β,16α,20α-triol (34 μg/d; RI: <10) [Table 1, Figs 1a, 2a-c]. The FM/THS ratio was 39 (RI: 96-390). Computed tomography (CT) showed a 7.9×5.6 cm left adrenal tumour, with calcification and possible compression on the left renal vein.

以尿中類固醇激素水平測試確診和監測腎上腺皮質癌

尿中類固醇激素水平測試有助確診和監測腎上腺皮質癌；不過，反映腎上腺癌的異常模式仍未明確。本文回顧五名腎上腺皮質癌患者於入院和隨訪期間的尿中類固醇激素水平，並將其結果與76名良性腎上腺皮質腺瘤患者和屬對照組的172人作比較。在因腫瘤入院和／或隨訪期間發現腫瘤殘留的患者中，發現三種異常尿類固醇排泄模式，包括：（1）多個類固醇軸出現分泌過多情況；（2）異常代謝物的分泌，尤以5-pregnene-3α,16α,20α-triol、5-pregnene-3β,16α,20α-triol，和後新生兒的新生兒類固醇代謝物為甚；（3）tetrahydro-11-deoxycortisol於總氫化皮質醇代謝物的比例上升。這些初步研究結果，有助以氣相色譜質譜法進行尿中類固醇激素水平測試，從而確診和監測腎上腺皮質癌。

At adrenalectomy, the tumour was partially fixed to the left renal vein. A radical left nephrectomy and left adrenalectomy were performed. A histological examination revealed cords and trabeculae with a rich vascular network and a diffuse growth pattern. Mitosis was frequent and vascular permeation and capsular invasion were observed. The overall histological picture was consistent with ACC, with tumour involvement of the raw surface, tumour thrombus in the left renal vein, and tumour invasion into adjacent adipose tissue. After operation, the clinical features of CS, hypokalaemia, and UFC level returned to normal. A follow-up USP done 6 months after surgery showed markedly decreased levels of FM compared with the preoperative sample. 5-Pregnene-3β,16α,20α-triol was no longer detectable, and 5-pregnene-3α,16α,20α-triol had also decreased to normal (16 μg/d). Nevertheless, her THS remained elevated (168 μg/d), with the FM/THS ratio remaining low, at 17. Computed tomography of thorax, abdomen and pelvis revealed suspicious lymph nodes in the perisplenic region. Positron emission tomography (PET) was negative. Three months later, multiple peritoneal and retroperitoneal nodules consistent with metastases were detected on CT. Chemotherapy in the form of cisplatinum and etoposide, gemcitabine and carboplatin, and thalidomide failed to arrest the disease progression. A follow-up USP revealed elevated steroid marker levels, with a high THS (1061 μg/d), 5-pregnene-3α,16α,20α-triol (281 μg/d), and 5-pregnene-3β,16α,20α-triol (48 μg/d). Her FM remained low (863 μg/d), with a FM/THS ratio of less than 1. She developed multiple intracranial haemorrhages 3 months later, possibly due to brain metastases, and died 28 months after surgery and 31 months after presentation.

Patient 2

This was a 58-year-old female patient who presented in May 2005 with hypertension of 195/115 mm Hg, hypokalaemia of 2.9 mmol/L, and increased urinary potassium excretion. She had no Cushingoid features, and no features of virilisation. Her UFC levels were

TABLE 1. Laboratory findings of adult patients with adrenocortical carcinoma (ACC)*†

Findings	ACC patient No.				Reference interval	
	1	2	3	4	Male	Female
Serum studies						
Spot serum cortisol level (nmol/L)	**546**	**645**	280	32.5	7-10 am: 171-536 4-8 pm: 64-340	
Erect plasma renin activity (ng/mL/h)	1.42	2.91	**<0.10**	3.48	0.97-4.18	
Erect aldosterone level (pmol/L)	317	**1469**	**>3300**	256	111-860	
DHEAS level (μmol/L)	1.4	**25.1**	3.8	Not done	2.2-15.2	0.9-11.7
ACTH level (pmol/L)	<2.2	<2.2	<2.2	<2.2	<10.1	
Urine free cortisol level (nmol/d)	**2019**	**3538**	193	28	100-379	
USP						
AM (μg/d)	778	1001	1371	1452	1047-5509	377-3205
DHAM (μg/d)	223	**44 403**	939	66	273-5255	98-3020
FM (μg/d)	**26186**	**8727**	**9397**	3431	3504-14 867	1906-7839
THS (μg/d)	**665**	**404**	**335**	83	10-90	9-59
5-Pregnene-3α,16α,20α-triol (μg/d)	**128**	**1872**	**209**	11	15-89	5-44
5-Pregnene-3β,16α,20α-triol (μg/d)	**34**	**1881**	**43**	<1	<51	<10
FM/THS ratio	**39**	**22**	**28**	**41**	116-542	96-390

* Serum and urine results of patient 4 were available only after removal of the primary tumour; data in bold are values that reflect increased steroid secretions

† ACTH denotes adrenocorticotropic hormone; AM total androstenedione metabolites; DHAM total dehydroepiandrosterone metabolites; DHEAS dehydroepiandrosterone sulphate; FM total cortisol metabolites; THS tetrahydro-11-deoxycortisol; and USP urinary steroid profiling

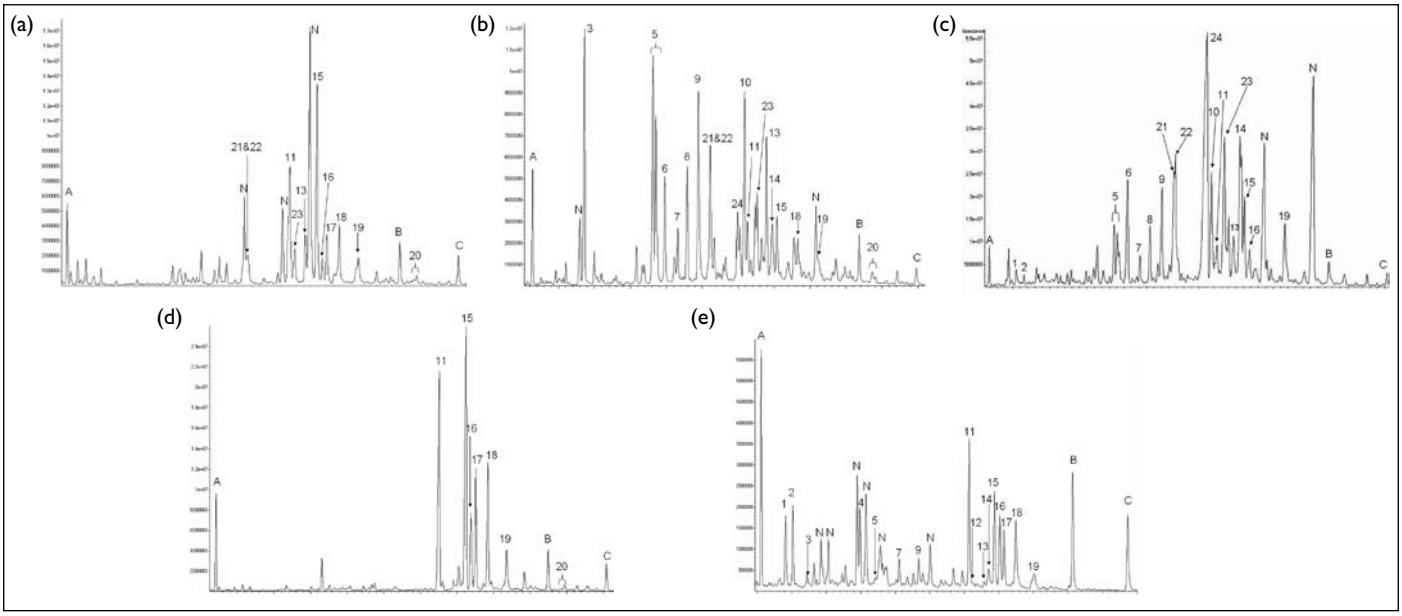

FIG 1. Urinary steroid profiling chromatograms of (a) patient 1, (b) patient 2, (c) patient 5, (d) a female patient with a cortisol-secreting adrenal adenoma, and (e) a healthy 56-year-old female volunteer

A: 5α-androstane-3α,17α-diol; B: stigmasterol; C: cholesteryl butyrate; 1: androsterone; 2: aetiocholanolone; 3: dehydroepiandrosterone; 4: 11-hydroxyandrosterone; 5: 16α-hydroxydehydroepiandrosterone; 6: pregnanediol; 7: pregnanetriol; 8: pregnanediol; 9: androstenetriol; 10: pregnanetriol; 11: tetrahydrocortisone; 12: tetrahydro-11-dehydrocorticosterone; 13: tetrahydrocorticosterone; 14: allo-tetrahydrocorticosterone; 15: tetrahydrocortisol; 16: allo-tetrahydrocortisol; 17: α-cortolone; 18: β-cortolone and β-cortol; 19: α-cortol; 20: cortisol; 21: tetrahydro-11-deoxycortisol; 22: 5-pregnene-3α,16α,20α-triol; 23: 5-pregnene-3β,16α,20α-triol; 24: 16α-hydroxypregnenolone; N: non-steroidal contaminants

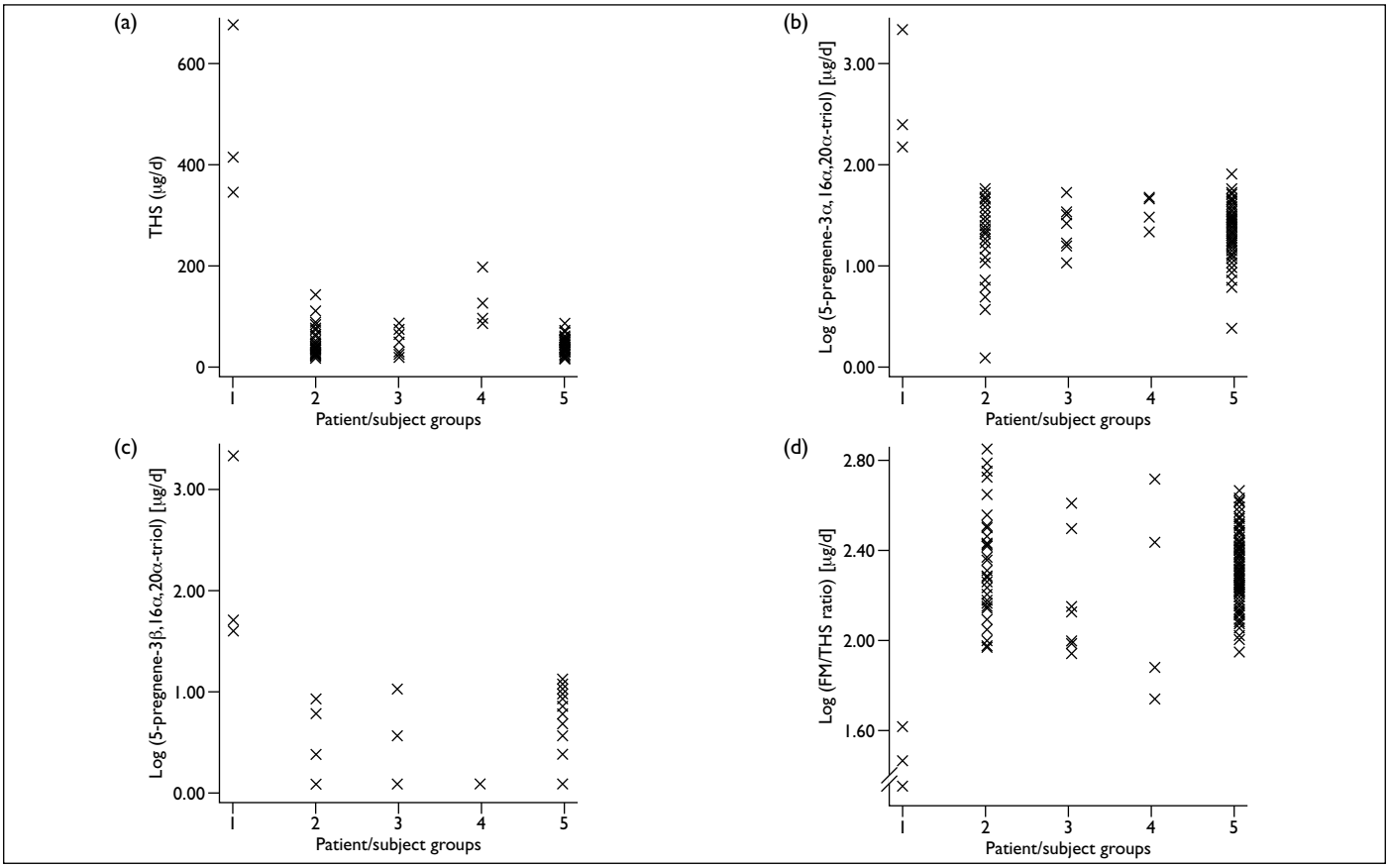

FIG 2. Levels of steroid metabolite excretion in different groups of adult female patients: (a) tetrahydro-11-deoxycortisol (THS); (b) 5-pregnene-3α,16α,20α-triol after log10; (c) 5-pregnene-3β,16α,20α-triol after log10; and (d) total cortisol metabolites (FM)/THS ratio after log10

Group 1: female patients with adrenocortical carcinoma; group 2: female patients with adrenal incidentalomas; group 3: female patients with Conn's syndrome; group 4: female patients with Cushing's syndrome; and group 5: female healthy volunteers. No 5-pregnene-3β,16α,20α-triol was detected in the two patients with Cushing's syndrome

3408 and 3538 nmol/d on two separate occasions. She had no diurnal cortisol rhythm, with morning and evening serum cortisol levels of 645 nmol/L and 615 nmol/L, respectively. After being given 1 mg overnight dexamethasone, her cortisol level was 559 nmol/L. The ACTH level was less than 2.2 pmol/L, and her DHEAS level was elevated at 25.1 μmol/L (RI: 0.9-11.7). Her erect PRA was 2.91 ng/mL/h (RI: 0.97-4.18), and aldosterone level 1469 pmol/L (RI: 111-860). Urinary steroid profiling detected marked increases in total DHEA metabolites (DHAM) and FM, as well as an excess of THS and 5-pregnene-3,16,20-triols (Table 1, Figs 1b, 2a-c). The FM/THS ratio was 22. She was also excreting high levels of 16α-hydroxypregnenolone, a neonatal steroid metabolite that is virtually absent in the post-neonatal period. Computed tomography showed an 8.0-cm right-sided tumour mass, with invasion into the inferior vena cava and possibly the right kidney. She also had multiple lung metastases and enlarged abdominal and hilar lymph nodes. She was given mitotane but failed to respond. Her general condition deteriorated rapidly, and she died 10 months after presentation. A post-mortem examination was not performed.

Patient 3

This was a 42-year-old female patient who presented with lower limb weakness due to hypokalaemia of 2.1 mmol/L in June 2003. Her blood pressure (BP) was 138/78 mm Hg. She had no Cushingoid features and no features of virilisation. Investigations confirmed urinary potassium loss and metabolic alkalosis. Her erect PRA was <0.10 ng/mL/h and her aldosterone level was >3300 pmol/L. A normal saline suppression test confirmed primary aldosteronism. Her serum and urinary cortisol levels and DHEAS were all normal. Quantification of her steroid metabolites, however, revealed an elevated excretion of FM. Her total androstenedione metabolites (AM) and DHAM levels were normal. She was also excreting excessive levels of THS, 5-pregnene-3α,16α,20α-triol, and 5-pregnene-3β,16α,20α-triol (335, 209, and 43 μg/d, respectively) [Table 1, Fig 2a-c]. Her FM/THS ratio was 28. Computed tomography showed a 5.7×5.3 cm left adrenal tumour with no evidence of internal fat. No local or distant invasion was evident. A left adrenalectomy was performed. A histological examination revealed a highly cellular tumour, which consisted of broad nests and diffuse sheets of moderately pleomorphic polygonal cells with round stippled nuclei, distinct nucleoli, multiple foci of necrosis, and mitoses of up to 11 per 10 high-power fields. Lymphovascular permeation was evident in the adjacent adrenal tissue. No capsular invasion was seen. The overall histological picture was consistent with ACC, stage T2.[8] After surgery her potassium, renin, and aldosterone levels returned to normal. Computed tomography, a PET scan, and her USP were also normal. She remained well at 52 months of follow-up.

Patient 4

This was a 24-year-old male patient who presented with recurrent lower abdominal pain in 2003. At operation for presumed appendicitis, an incidental abdominal mass was found and resected. On pathological examination it was found an 8.2×6.2 cm adrenal tumour with moderate nuclear pleomorphism and distinct nucleoli, localised foci of coagulative necrosis and vascular invasion, compatible with ACC. When referred to us, his BP was 110/62 mm Hg, and a physical examination revealed left gynaecomastia only. His potassium level was normal. His UFC level was 28 nmol/d and his DHEAS, renin and aldosterone levels were normal. His total testosterone level was 14.6 nmol/L, and oestradiol level 94 pmol/L. His USP was normal, though the THS was on the high side (83 μg/d, RI for males: 10-90) and his FM/THS ratio was low (41; RI: 116-542) [Table 1, Fig 2a-c]. His 5-pregnene-3,16,20-triol levels were not elevated. Computed tomography showed absence of a right adrenal and a normal left adrenal gland. Two suspicious hypodense lesions were detected in the dome and segment 6 of his liver. Positron emission tomography and fine-needle aspiration of the liver lesions were negative. An exploratory hepatectomy revealed metastatic ACC. Computed tomography performed 4 months later revealed another lesion in segment 4 of the liver and PET became positive 3 months afterwards. The patient underwent further debulking surgery, followed by adjuvant chemotherapy. Use of mitotane, cisplatinum and etoposide or gemcitabine, and thalidomide failed to control his disease. He died 28 months after presentation.

Patient 5

This was a Pakistani girl who presented in September 2004 with recurrent convulsions and impaired consciousness caused by hypertensive encephalopathy at the age of 25 months. Her BP was persistently around 160/110 mm Hg, and she had increased body hair, pubic hair growth, progressive abdominal distension, and a marked increase in appetite and body weight from the age of 18 months. Physical examination revealed marked hirsutism, acne, a moon face, truncal obesity, and striae over her abdomen. A firm abdominal mass was palpable 3 cm below the left costal margin. Her BP was controlled with a labetolol infusion. She had severe hypokalaemia of 1.9 mmol/L but her serum sodium and creatinine levels were normal. Her cortisol levels were 856 nmol/L in the morning and 696 nmol/L at night. Her testosterone level was 19.1 nmol/L (RI: 0.1-0.6), oestradiol level 183 pmol/L (RI: 22.0-99.1), and

progesterone level 61.9 nmol/L (RI: 0.22-1.65). Her urine catecholamines were normal. Urinary steroid profiling revealed a significant increase in AM (378 μg/d; RI: <101), FM (7458 μg/d; RI: 389-2730), corticosterone (3260 μg/d; RI: 33-409), pregnanediol (1541 μg/d; RI: <29), THS (1067 μg/d; RI: <49), 5-pregnene-3α,16α,20α-triol (827 μg/d; RI: <17), and 5-pregnene-3β,16α,20α-triol (930 μg/d; RI: <2). The concentration of 16α-hydroxypregnenolone was highest among all steroid metabolites (Fig 1c). Computed tomography of her abdomen showed a large left supra-renal mass suggestive of an adrenal tumour. Complete resection of the mass was performed. Histology confirmed ACC. She was put on hydrocortisone replacement and maintained satisfactory growth. Unusual steroid metabolites were no longer detectable 4 months and 2 years after the operation.

Patients with adrenal adenoma

The clinical and laboratory data of 83 patients with adrenal nodules who had USP performed between 2003 and 2007 were retrieved for comparison. Of these, seven subjects were excluded from analysis: four had congenital adrenal hyperplasia, one had adrenal lymphoma, one had pituitary Cushing's disease, and one presented with clinical features of primary hyperaldosteronism but defaulted follow-up after 1 month. All those adenomas found to be functional after biochemical investigations were resected, and the diagnosis of benign functional ACA confirmed by pathology and clinical improvement after operation. Patients in whom the diagnosis of 'incidentaloma' was made because of negative biochemical findings had at least one repeated CT after 1 year that showed no increase in the size of the adrenal nodule(s). Of the 76 patients with benign ACA, 57 had incidentaloma, 16 had primary aldosteronism, two had adrenal CS, and one had subclinical CS; 35 were male and 41 female patients. Their age ranged from 34 to 87 years, with a mean of 57 years. In only three was the adrenal nodule ≥4 cm in diameter (4.0, 4.5, and 4.6 cm). Their clinical and laboratory findings are listed in Tables 2 and 3. Figure 1d shows the urine steroid gas chromatogram of a female patient with CS. There was a gross elevation in the level of excretion of FM; AM were virtually absent. A gas chromatogram of a healthy 56-year-old female subject is shown in Figure 1e for comparison. Figure 2 shows the levels of excretion of THS, 5-pregnene-3,16,20-triols, and the FM/THS ratio in different groups of adult female patients and the healthy volunteers. Patients with ACC had the highest excretion levels of these metabolites (cases 1 to 3) among all the groups of patients with adrenal tumours and sex-matched healthy subjects (Fig 2a-c). The log (FM/THS) ratio in all the female ACC patients was lower than all other groups of subjects studied (Fig 2d). The male data are listed in Table 3.

None of the subjects were taking steroids or hormonal pills during the urine and serum studies. Verbal consent for the investigations was given by all subjects.

Urinary steroid profiling

The methodology used for USP and the adult RI of steroid metabolites have been described by our group previously.[9] Reference intervals for girls of less than 6 years were derived from USP data from nine healthy age-matched control subjects. The AM excretion was defined as the sum of androsterone and aetiocholanolone. The DHAM excretion was defined as the sum of DHEA, 16α-hydroxydehydroepiandrosterone, and androstenetriol. The FM excretion was defined as the sum of tetrahydrocortisone, tetrahydrocortisol (THF), 5α-THF, α-cortolone, β-cortolone, β-cortol, and α-cortol.

Discussion

Endocrinology services are receiving an increasing number of patients referred for investigations after the incidental discovery of an adrenal nodule.[1] Along with investigating hormonal hyperfunction, it is also important to consider ACC so it can be identified early. The only means of achieving long-term survival of this highly aggressive condition is early detection and radical surgical extirpation.[10] Unfortunately, like most endocrine malignancies, differentiating between benign and malignant adrenal tumours can be difficult before metastases develop, even with histological examination.[11] Clues that should alert clinicians to the possibility of ACC include a tumour size of greater than 4 to 5 cm in diameter, imaging features of calcification, blurred margins, an irregular shape, heterogeneous contrast enhancement and distant metastases on CT, a high signal intensity on MRI T2-weighted images, negative uptake on an iodocholesterol scan, a positive uptake on an 18F-FDG PET scan, and hormonal overproduction in more than one adrenocortical axis.[12-15] Hormone secretion was noted in up to 79% of ACC.[5,16] In this article, we focus our discussion on the value of USP as a tool for differentiating ACC from benign adenomas.

Determination of the USP has been proposed as a useful tool for the detection and follow-up of ACC[5,17] but because of the low prevalence of ACC, the abnormal patterns characterising this condition and the best way to utilise this tool have not yet been determined. High levels of a range of steroid metabolites have been reported in ACC: pregnanediol (a metabolite of pregnenolone), pregnanetriol (a metabolite of 17-hydroxypregnenolone), THS (metabolite of 11-deoxycortisol), androstenetriol (a metabolite of DHEA), and 5-pregnanetriol.[4-6,17,18]

Our data show that USP may assist with the

TABLE 2. Clinical findings of adult patients with adrenocortical carcinoma (ACC) and adrenocortical adenoma (ACA)*

Clinical findings	ACC patient No.				ACA patients (n=76)	Normal (n=172)
	1	2	3	4		
Age at presentation (years) Mean±standard deviation (range)	51 -	58 -	42 -	24 -	- 57±14 (34-87)	- 43.1±13.1 (20-85)
Sex	Female	Female	Female	Male	41 Females, 35 males	89 Females, 83 males
Lateralisation of adrenal nodule	Left	Right	Left	Right	34 Left, 38 right, 4 bilateral	NA
Size of adrenal nodule (cm)†	7.9	8.0	5.7	8.2	73 were <4 cm, 3 were ≥4 cm	NA
Endocrine syndrome at presentation	Cushing's	Conn's	Conn's	Nil	3 Cushing's, 16 Conn's, and 57 incidentaloma	NA
Duration of follow-up (months)	31 (Died)	10 (Died)	52	28 (Died)	Median 34, IQR 24.5-47	

* NA denotes not applicable, and IQR interquartile range
† Maximum diameter on imaging studies

TABLE 3. Laboratory findings of patients with adrenocortical adenoma (ACA)*

Laboratory findings		ACA patients			Reference interval
		Cushing's syndrome (male=1†, female=2)	Conn's syndrome (male=8, female=8) [mean±SD]	Incidentaloma (male=26, female=31) [mean±SD]	
Serum/plasma/urine studies					
Spot serum cortisol level (nmol/L)	Male Female	307 766, 787	272±142 257±74	343±273 341±130	7-10 am: 171-536 4-8 pm: 64-340
Erect plasma renin activity (ng/mL/h)	Male Female	ND ND	0.81±0.80 0.21±0.22	2.12±1.85 (n=16) 2.23±2.30 (n=17)	0.97-4.18
Erect aldosterone level (pmol/L)	Male Female	ND ND	751±464 1554±1181	243±123 (n=19) 445±390 (n=18)	111-860
DHEAS level (μmol/L)	Male Female	3.2 <0.5, ND	2.0, 7.8 (n=2) 0.7 (n=1)	3.0±2.2 (n=13) 1.6±0.9 (n=11)	2.2-15.2 0.9-11.7
ACTH level (pmol/L)	Male Female	<2.2 <2.2, <2.2	ND	4.4±2.3 (n=13) 3.9±2.2 (n=11)	<10.1
Urine free cortisol level (nmol/d)	Male Female	237 789, 1331	235±75 185±100	201±92 167±68	100-379
USP					
AM (μg/d)	Male Female	1232 104, 106	1661±564 813±391	1499±955 868±903	1047-5509 377-3205
DHAM ((μg/d)	Male Female	1360 52, 133	892±795 496±325	601±451 482±998	273-5255 98-3020
FM (μg/d)	Male Female	17 302 26 685, 9881	8791±3817 4935±1974	9567±6167 6901±3178	3504-14 867 1906-7839
THS (μg/d)	Male Female	70 55, 189	48±20 40±24	52±35 38±29	10-90 9-59
5-Pregnene-3α,16α,20α-triol (μg/d)	Male Female	54 11, 40	31±16 21±11	35±23 18±13	15-89 5-44
5-Pregnene-3β,16α,20α-triol (μg/d)	Male Female	4 0, 0	4±3 2±3	4±5 1±2	<51 <10
FM/THS ratio	Male Female	246 487, 52	206±102 163±112	214±114 248±158	116-542 96-390

* ACTH denotes adrenocorticotropic hormone; AM total androstenedione metabolites; DHAM total dehydroepiandrosterone metabolites; DHEAS dehydroepiandrosterone sulphate; FM total cortisol metabolites; ND not done; SD standard deviation; THS tetrahydro-11-deoxycortisol; and USP urinary steroid profiling
† The male patient had subclinical Cushing's syndrome

diagnosis of ACC in three ways. Firstly, it reveals the presence of hormonal overproduction in more than one adrenocortical axis, as exemplified by patients 1, 3, and 5. Urinary steroid profiling includes metabolites not measured in conventional serum tests. In addition, 24-hour urine collections are less affected by diurnal variations and episodic secretions, and are thus a more sensitive method of detecting abnormal levels. In this aspect, USP offered less additional information in patients 2 and 4 because serum studies already showed hormonal overproduction in more than one axis in patient 2, while neither serum nor USP measurements showed elevated levels in any axis in the latter. Patient 4 was referred after surgery so the investigations were done in the absence of the primary adrenal tumour. Although he still had liver metastases, dedifferentiation of the metastasised tissue may have led to normalisation of a previously abnormal pattern of hormone production or metabolism.

Secondly, USP is useful for revealing production of unusual metabolites such as 5-pregnene-3α,16α,20α-triol, 5-pregnene-3β,16α,20α-triol or 16α-hydroxypregnenolone in ACC, as seen in our patients. These metabolites are unusual in the sense that, though low levels were detectable in our healthy subjects, they were not elevated in any of our patients with benign adrenal nodules, be these functional or incidental tumours. Indeed, both 5-pregnene-3α,16α,20α-triol and 5-pregnene-3β,16α,20α-triol were first isolated from ACC patients.[19,20] The structure of these two compounds is based on chromatographic retention time as well as the infrared spectrum. A USP chromatogram as shown in Figure 1 allows an overall appreciation of grossly abnormal excretion of particular steroids and steroid metabolites, enabling unusual metabolites to be identified. This property is particularly useful in conditions such as ACC, in which relative enzyme deficiencies in the steroid metabolic pathway may lead to the production of unusual steroid metabolites.[21]

Thirdly, USP may assist with the diagnosis of ACC by enabling detection of subtle abnormalities in the quantities of different metabolites. Since THS is the metabolite of one of the intermediates (11-deoxycortisol) in the cortisol pathway, it was not surprising to find elevated THS in ACC patients and in patients with CS in whom FM was elevated (Fig 2a). Previous reports on ACC have also noted that THS is a useful tumour marker.[4-6] We observed that in ACC, THS appeared to be disproportionately high compared with other cortisol metabolites, as illustrated by the marked decrease in the FM/THS ratio (Fig 2d). The decrease in this ratio was less dramatic in patients with CS. Indeed, in patient 4 in whom we could only perform USP after removal of the primary adrenal tumour, suppression of the FM/THS was the only abnormality identified.

Urinary steroid profiling can also be used to indicate incomplete removal or relapse of ACC, as illustrated by patient 1. In this patient, her cushingoid features and UFC levels returned to normal after surgery, but her urine THS and the FM/THS ratio remained abnormal. Her subsequent course confirmed residual disease. At the terminal stage of her disease, a rise in THS and 5-pregnene-3,16,20-triols, and a fall in her FM/THS ratio informed us of her disease progression. In this patient, the USP pattern of residual disease was similar to that before treatment, but change in the secretion pattern has been described as the tumour or its metastases change in size, growth rate and differentiation.[5] The value of USP for monitoring ACC was supported by Khorram-Manesh et al.[17] Among five of their patients who had both pre- and post-operative urine samples, residual or recurrent ACC was identified in two because of abnormalities in their USP despite normal imaging studies. Because age- or sex-related normal values for USP using GC-MS had not been established at the time of their study, these investigators compared postoperative with preoperative samples from the same patient.

All five of our patients with ACC had some CT features of adrenal carcinoma, most notably large tumours, raising the question whether USP really played an additional role in the diagnosis of this condition. Nevertheless, there is no clear CT feature—apart from the demonstration of metastases or adjacent tissue invasion—that is diagnostic of adrenal carcinoma, so we still have to rely on a conglomeration of data for earlier diagnosis. This includes clinical, radiological as well as biochemical data, and USP contributes by providing a comprehensive biochemical assessment of adrenal secretions. In addition, in public health care, the waiting time for a CT scan can range from 3 to 6 months, which is very long if one takes into consideration the poor prognosis of adrenal carcinoma. An abnormal USP can alert clinicians to arrange earlier CT scanning and earlier surgery for the patient. In fact, this was the case with one of our patients. Urinary steroid profiling may also warn clinicians of residual disease after surgery, before it can be definitively identified on CT.

In conclusion, our experience supports the notion that USP can be useful in differentiating ACC from ACA. Our data suggest that we can focus our attention on three aspects: (1) hormonal hypersecretion in multiple axes, (2) excretion of unusual metabolites, and (3) subtle alterations in the metabolic pathways. A low FM/THS ratio and elevated levels of 5-pregnene-3,16,20-triols are potential tumour markers for ACC. Because of the small number of ACC subjects in our study, we can only describe our findings as preliminary. We cannot define the role of USP in the diagnosis and management of ACC at this stage, but we hope to arouse clinicians'

awareness of this tool, so that more experience can be accumulated in the future. Adrenocortical carcinoma is a rare, highly malignant yet curable disease whose preoperative identification by clinical and imaging features is difficult. Thus, it is important to make a concerted effort to document the role of different investigation modalities in differentiating between benign and malignant adrenal nodules.

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