Urinary cortisol level in exclusion autonomous cortisol secretion in the patient of primary aldosteronism

Wen-Kai Chu  
National Taiwan University Hospital

Chih-Yuan Wang  
National Taiwan University Hospital

Wan-Chen Wu  
National Taiwan University Hospital

Vin-Cent Wu (✉️ q91421028@ntu.edu.tw)  
National Taiwan University Hospital

Research Article

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Abstract

Background

Serum cortisol level after 1-mg overnight dexamethasone suppression test (1-mg DST) below 1.8 ug/dL was a diagnostic criterion for having autonomous cortisol secretion (ACS), whether the cut-off point in 24-hour urine-free cortisol (24-h UFC) for the patient suspecting with primary aldosteronism (PA) concomitant ACS is unclear.

Methods

This prospective observational study enrolled 274 patients diagnosed with PA from January 2017 to January 2020 (male, 42.3%; mean age, 55.9 ± 11.7 years). Serum cortisol level after 1 mg DST over 1.8 ug/dL was a diagnostic criterion for ACS, confirmed with a second repeated test.

Results

Of the 274 PA patients, 74 patients (27%) with PA had concomitant ACS while the other 200 patients were not. Logistic regression analysis showed patients with PA concomitant ACS were associated with higher 24-h UFC (OR, 1.91 [95% CI, 1.06–3.41], P=0.03), older age (OR, 1.04 [95% CI, 1.01–1.07], P=0.008), and diabetes mellitus (OR, 2.4 [95% CI, 1.12–5.12], P=0.025). The generalized additive model (GAM) for urinary cortisol and ACS showed the 24-h UFC above 36 μg, concurrent with the positive predictive value of 32.6% and negative predictive value of 77.9% could be a factor predicting a higher possibility of ACS.

Conclusions

More than a quarter of PA patients concomitant ACS. Our study suggested the 24-h UFC less than 36 μg as a cut-off point in exclusion of the patient with PA concomitant ACS. Additionally, older age and diabetes mellitus were also risk factors for predicting patients with PA concomitant ACS.

Background

Previous studies have reported a rising prevalence of concomitant autonomous cortisol secretion (ACS) among patients with primary aldosteronism (PA), ranging from 12.8–33.3% [1, 2]. ACS in patients with PA has the subtle cortisol hypersecretion from the adrenal tumor without specific symptoms and signs of overt hypercortisolism [3]. ACS was used to be diagnosed in patients of adrenal incidentaloma, those who were not diagnosed with Cushing syndrome but the biochemical tests showed that there was autonomic hypersecretion of cortisol [4]. Although in patients with ACS, even mild increased cortisol levels may contribute to associated metabolic risks such as diabetes and hypertension, and may increase the risk of cardiovascular events and mortality [5, 6]. Concomitant ACS in patients with PA was associated with a higher prevalence (21.6%) of diabetes mellitus than in the general population (12.1%) as well as severe left ventricular hypertrophy, lowered estimated glomerular filtration rate (eGFR) and proteinuria [2, 7, 8].
The diagnostic methods and cut-off points for ACS in patients with adrenal tumors were inconsistent. 1-mg overnight dexamethasone suppression test (1-mg DST) is currently the golden standard, which is also suggested to be performed on the patient with adrenal incidentaloma according to the 2016 European Network for the Study of Adrenal Tumors (ENSAT) adrenal tumors clinical practice guideline [4]. Most endocrinologists agree that a serum cortisol level of at least 1.8 ug/dL after a 1-mg dexamethasone suppression test (DST) is a diagnostic indicator for ACS. [4, 9].

For patients with ACS, 24-hour urine-free cortisol (24-h UFC) can be measured to assess whether there is excessive cortisol secretion. However, there were no cut-off points in 24-h UFC for the patient suspecting PA concomitant ACS. The purpose of this study was to prospectively examine the relationship between 24-h UFC and ACS in a prospective enrolled PA cohort.

**Methods**

**Patients**

The Taiwan Primary Aldosteronism Investigation (TAIPAI) registry was a prospectively designed multicenter cohort, recruiting patients with a suspected PA diagnosis. The database was constructed to ensure quality control at five regional hospitals and two medical centers located in various cities throughout Taiwan. Patients with other secondary hypertension, including, but not limited to, renovascular hypertension, Cushing’s syndrome, hyperthyroidism, and pheochromocytoma, were all excluded from this study. All anti-hypertensive medications were discontinued for at least 21 days before PA screening tests. Doxazosin and/or diltiazem were administered to control markedly high blood pressure when required. Patients who were confirmed with family type I (FH-I)/ glucocorticoid-remediable aldosteronism were excluded from the analysis via long-range polymerase chain reaction, as described previously.

Ethical approval (approval number 200611031R) was obtained from the institutional review board of the National Taiwan University Hospital. Written informed consent for clinical data collection and research use was obtained from all participants before enrollment in the study. The screening confirmation was performed according to standard published protocols [10].

In this study, we enrolled 274 patients diagnosed with PA from January 2017 to January 2020 in the TAIPAI database and we retrospectively analyzed the patients’ clinical information and demographic data. This study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of National Taiwan University Hospital (200611031R, 201901114RIND). Before being included in the study, all participants provided their informed consent.

**PA identification**

The diagnosis and subtype of PA were established in hypertensive patients as follows. PA was diagnosed based on fulfilling the following three conditions [11, 12]: (1) autonomous excess aldosterone production
as indicated by an aldosterone-renin ratio (ARR) greater than 35 (ng/dL)/(ng/mL/h); (2) a TAIPAI score > 60% [13]; (3) plasma aldosterone concentration (PAC) > 16 ng/dL [14] post-saline loading test, or ARR > 35 (ng/dL)/(ng/mL/h) after a captopril or losartan test [15].

We used a criteria of autonomous excess aldosterone production evidenced with ARR > 35 ng/dL per ng/ml/hr after captopril test, TAIPAI score > 60%

# The probability of PA (TAIPAI score) was equal to:

$$P = 1 + e^{-\beta}$$

where $$\beta = (\text{PAC}[\text{ng/dl}] \times [0.063]) + \text{PRA}[\text{ng/ml/h}] \times [-0.205]) + ([\text{ARR} \times 0.001] \times [\text{BMI} [\text{kg/m}^2] \times [0.067]) + (\text{Male} \times [-0.738] + \text{SK} [\text{mmol/l}] \times [-1.512]) + (\text{eGFR} [\text{ml/min/1.73 m}^2] \times [0.017]) + ([\text{propensity score}] \times [-0.539] + [1.851])$$

(Abbreviations: PAC, plasma aldosterone concentration; PRA, plasma renin activity)

The following criteria were used to diagnose unilateral primary aldosteronism (uPA) [15]: (1) the presence of an adrenal nodule or thickening observed through a computer tomography scan; (2); lateralization of aldosterone over-secretion in adrenal vein sampling; (3); classical aldosterone-producing adenoma, which was confirmed through pathological analysis. (APA; ≥10 mm in diameter), or aldosterone-producing nodule (APN; < 10 mm), or non-classical multiple positive CYP11B2-stained APNs or aldosterone-producing micronodules (mAPN/mAPM) in immunohistochemistry according to the HISTALDO consensus [16, 17] if the patient underwent adrenalectomy.

**Clinical and biochemical measurements**

Baseline demographic characteristics included age, sex, body mass index (BMI), waist-to-hip ratio (WHR), comorbid conditions, systolic and diastolic blood pressure, and heart rate. Baseline plasma aldosterone concentration (PAC) and plasma renin activity (PRA) were documented using specific radioimmunoassay kits (ALDO-RIACT RIA kit, Cisbio Bioassays, Codolet, France and Ang I RIA Kit, Beckman Coulter, Immunotech, Prague, Czech Republic). Serum creatinine, potassium, intact parathyroid hormone (iPTH), and 24-h UFC were also measured. Cortisol levels were measured using a chemiluminescent enzyme immunoassay (Archeitect, Abbott, VA, USA), and ACTH levels were measured using an electrochemiluminescent immunoassay (Immulate 2000, Diagnostic Products Corporation, Los Angeles, CA, USA).

**Assessment of ACS**

Endocrinologists commonly use overnight 1-mg dexamethasone suppression test (DST) as a rigorous diagnostic criterion for concomitant autonomous cortisol secretion (ACS). This involves administering 1 mg dexamethasone orally to the patient around 11 PM, followed by a single blood sample taken at 8 AM the following morning to measure the serum cortisol level. Post-DST cortisol levels of 1.8 µg/dL or higher are used as the cut-off point. [4, 9]. Despite the existence of several guidelines, there is no consensus on the biochemical diagnosis of concomitant autonomous cortisol secretion (ACS). Saliva cortisol was not
checked as it cannot replace serum cortisol to identify ACS using the DST [18]. Some studies have utilized suppressed adrenocorticotropic hormone (ACTH), increased urinary free cortisol, and elevated midnight cortisol as criteria for ACS. However, due to the lack of standardization of these criteria across studies, we chose to define ACS only based on the results of the overnight 1-mg dexamethasone suppression test (DST) [19]. In order to prevent ascertainment bias, a second positive overnight 1-mg dexamethasone suppression test (DST) was required to confirm the presence of ACS. Patients who had received estrogen or steroid therapy, those with a body mass index (BMI) of 30 kg/m2 or higher, and those with a history of excessive alcohol intake were excluded from the study [20].

**Statistical analysis**

We reported frequencies and percentages for categorical variables, and mean with standard deviation (SD) for continuous variables. To compare differences between categorical variables, we used the \( \chi^2 \) test, two-sample t-test, and Mann–Whitney U-test as indicated. In the regression analysis, we included age, sex, diabetes mellitus, systolic blood pressure, diastolic blood pressure, PAC, PRA, and 24-h urine-free cortisol. A generalized additive model (GAM) was applied to assess the associations between urinary cortisol against the odds risk of ACS adjusting age, sex, systolic blood pressure, diastolic blood pressure, PAC, and PRA. We defined the optimal cortisol cut-off value as log [odds ratio] (OR) equaling zero [21–23]. We considered a two-sided P value of \( \leq 0.05 \) to be statistically significant.

We used STATA/SE 14.0 for Windows (StataCorp LP, College Station, TX, USA) and R software version 3.4.4 (Free Software Foundation, Inc., Boston, MA, USA) to perform the statistical analyses.

**Results**

In total, 274 PA patients were enrolled in this study. Their mean age was 55.9 ± 11.7 years and 116 (42.3%) were male. The mean baseline eGFR was 92.2 ± 25.6 mL/min/1.73m2. Of the 274 PA patients, 74 patients (27.0%) with PA had concomitant ACS by the criteria of 1-mg DST while the other 200 patients were not. Table 1 summarizes the clinical variables comparison of the two groups.
Table 1  
Baseline characteristics of patients with PA concomitant autonomous cortisol secretion

<table>
<thead>
<tr>
<th></th>
<th>ALL</th>
<th>ACS(+)</th>
<th>ACS(-)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 274</td>
<td>n = 74</td>
<td>n = 200</td>
<td></td>
</tr>
<tr>
<td>Age, year</td>
<td>55.9 ± 11.7</td>
<td>59.4 ± 11.0</td>
<td>54.6 ± 11.6</td>
<td>0.002*</td>
</tr>
<tr>
<td>Male sex</td>
<td>116 (42.3%)</td>
<td>29 (39.2%)</td>
<td>87 (43.5%)</td>
<td>0.521</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.6 ± 4.6</td>
<td>25.5 ± 4.3</td>
<td>25.7 ± 4.7</td>
<td>0.794</td>
</tr>
<tr>
<td>Waist to hip ratio (%)</td>
<td>89.1 ± 8.0</td>
<td>89.9 ± 8.4</td>
<td>88.9 ± 7.8</td>
<td>0.372</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>43 (15.7%)</td>
<td>19 (25.7%)</td>
<td>24 (12%)</td>
<td>0.006*</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>16 (5.8%)</td>
<td>1 (1.4%)</td>
<td>15 (7.5%)</td>
<td>0.078</td>
</tr>
<tr>
<td>Plasma aldosterone ...ng/dL</td>
<td>† 37.7 ± 25.4</td>
<td>37.8 ± 24.7</td>
<td>37.6 ± 25.7</td>
<td>0.960</td>
</tr>
<tr>
<td>Plasma renin activity †, ng/mL/h</td>
<td>1.0 ± 1.5</td>
<td>0.9 ± 1.4</td>
<td>1.0 ± 1.6</td>
<td>0.526</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>151.7 ± 22.9</td>
<td>154.5 ± 28.1</td>
<td>150.7 ± 20.8</td>
<td>0.297</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>90.3 ± 14.1</td>
<td>90.6 ± 16.9</td>
<td>90.2 ± 13.0</td>
<td>0.865</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>74.7 ± 12.0</td>
<td>72.7 ± 10.9</td>
<td>75.4 ± 12.3</td>
<td>0.103</td>
</tr>
<tr>
<td>Serum blood urea nitrogen, mg/dL</td>
<td>15.5 ± 7.9</td>
<td>17.4 ± 11.9</td>
<td>14.8 ± 5.7</td>
<td>0.090</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.9 ± 0.7</td>
<td>1.1 ± 1.2</td>
<td>0.9 ± 0.3</td>
<td>0.136</td>
</tr>
<tr>
<td>Serum potassium, mEq/L</td>
<td>3.9 ± 0.5</td>
<td>3.9 ± 0.6</td>
<td>3.9 ± 0.5</td>
<td>0.683</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73m²</td>
<td>92.2 ± 25.6</td>
<td>89.5 ± 28.9</td>
<td>93.3 ± 24.2</td>
<td>0.284</td>
</tr>
<tr>
<td>Serum cortisol, µg/dL</td>
<td>11.1 ± 3.9</td>
<td>12.0 ± 4.2</td>
<td>10.7 ± 3.8</td>
<td>0.016*</td>
</tr>
<tr>
<td>ACTH, pg/mL</td>
<td>23.0 ± 16.0</td>
<td>17.0 ± 12.4</td>
<td>25.2 ± 16.6</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>iPTH, pg/mL</td>
<td>71.9 ± 58.9</td>
<td>80.5 ± 98.0</td>
<td>68.6 ± 34.1</td>
<td>0.319</td>
</tr>
<tr>
<td>Urine free cortisol, µg/24hr</td>
<td>43.1 ± 30.6</td>
<td>47.1 ± 33.0</td>
<td>41.6 ± 29.6</td>
<td>0.183</td>
</tr>
<tr>
<td>Serum midnight cortisol level, µg/dL</td>
<td>7.5 ± 3.3</td>
<td>8.1 ± 2.5</td>
<td>7.2 ± 3.6</td>
<td>0.122</td>
</tr>
</tbody>
</table>

**Abbreviations:** ACTH, Adrenocorticotropic hormone; eGFR, estimated glomerular filtration rate; iPTH, intact parathyroid hormone

*P < 0.05, ***P < 0.001.

† Data after holding the medications that will interfere the renin-angiotensin-aldosterone system.
A serum cortisol concentration > 1.8 μg/dL after an overnight 1-mg DST without any overt Cushing features confirms the diagnosis of autonomous cortisol secretion. [4]

The patients with ACS were older (P = 0.002), had a higher prevalence of diabetes mellitus (P = 0.006), and had lower ACTH (P < 0.001) but higher serum cortisol levels (P = 0.016). The other clinical variables, such as sex, body mass index (BMI), blood pressure, and the prevalence of coronary artery disease, were similar between both groups.

In addition to that, we conducted an examination of the immunohistochemistry in the surgical cases, and found that the prevalence of CYP11B1 staining and DHEA-sulfotransferase was comparable between the two groups.

**The cut-off value of urinary cortisol level for ACS**

A generalized additive model (GAM) for urinary cortisol (Fig. 1) was applied to understand aggression against the probability of ACS. The 24-h UFC above 36 μg could be a factor predicting a higher possibility of ACS. The Sankey diagrams (Fig. 2) illustrates the percentages of primary aldosteronism patients with or without autonomous cortisol secretion and the 24-h UFC after follow-up.

The patients were divided into two groups based on daily urinary cortisol ≥ 36 μg (Table 2). 129 patients had urinary cortisol ≥ 36 μg while the other 145 patients had less urinary cortisol. In our study, a 24-h UFC cut-off point of 36 μg had the value in predicting patients that are possible ACS with a sensitivity of 56.8%, specificity of 56.5%, positive predictive value (PPV) of 32.6%, and negative predictive value (NPV) of 77.9%. Among the patient with higher urine-free cortisol, they had a higher percentage of males (P = 0.002). They also had higher serum cortisol levels before (P = 0.002) and after 1-mg DST (P = 0.002) but lower iPTH (P = 0.047). We observed that patients with elevated urine-free cortisol levels also had higher midnight cortisol levels (P = 0.035). However, we found no significant differences in the other clinical variables, such as the prevalence of CYP11B1 staining and DHEA-sulfotransferase, between the two groups.
Table 2
Baseline characteristics of patients with daily urinary free cortisol above and under 36 µg

<table>
<thead>
<tr>
<th></th>
<th>24-hr urine free cortisol ≥ 36µg, n = 129</th>
<th>24-hr urine free cortisol &lt; 36µg, n = 145</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>55.9 ± 11.9</td>
<td>55.8 ± 11.5</td>
<td>0.954</td>
</tr>
<tr>
<td>Male sex</td>
<td>67(51.9%)</td>
<td>49(33.8%)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.6 ± 3.7</td>
<td>25.7 ± 5.2</td>
<td>0.805</td>
</tr>
<tr>
<td>Waist to hip ratio (%)</td>
<td>90.0 ± 8.1</td>
<td>88.4 ± 7.8</td>
<td>0.106</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>23(17.8%)</td>
<td>20(13.8%)</td>
<td>0.359</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>8(6.2%)</td>
<td>8(5.5%)</td>
<td>0.810</td>
</tr>
<tr>
<td>Plasma aldosterone concentration†, ng/dL</td>
<td>39.8 ± 27.7</td>
<td>35.7 ± 23.0</td>
<td>0.187</td>
</tr>
<tr>
<td>Plasma renin activity†, ng/mL/hr</td>
<td>1.1 ± 1.5</td>
<td>0.9 ± 1.5</td>
<td>0.422</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>153.2 ± 23.7</td>
<td>150.4 ± 22.2</td>
<td>0.327</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>91.2 ± 15.1</td>
<td>89.5 ± 13.0</td>
<td>0.328</td>
</tr>
<tr>
<td>Heart rate, beats per minute</td>
<td>74.7 ± 12.8</td>
<td>74.7 ± 11.2</td>
<td>0.985</td>
</tr>
<tr>
<td>Serum blood urea nitrogen, mg/dL</td>
<td>14.8 ± 5.2</td>
<td>16.2 ± 9.7</td>
<td>0.138</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.9 ± 0.8</td>
<td>0.9 ± 0.7</td>
<td>0.965</td>
</tr>
<tr>
<td>Serum potassium, mEq/L</td>
<td>3.9 ± 0.5</td>
<td>3.9 ± 0.5</td>
<td>0.749</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73m²</td>
<td>95.4 ± 26.3</td>
<td>89.4 ± 24.7</td>
<td>0.056</td>
</tr>
<tr>
<td>Serum cortisol, µg/dL</td>
<td>11.9 ± 3.8</td>
<td>10.4 ± 3.9</td>
<td>0.002*</td>
</tr>
<tr>
<td>ACTH, pg/mL</td>
<td>23.9 ± 15.9</td>
<td>22.1 ± 16.0</td>
<td>0.365</td>
</tr>
<tr>
<td>iPTH, pg/mL</td>
<td>64.3 ± 50.6</td>
<td>78.5 ± 64.7</td>
<td>0.047*</td>
</tr>
<tr>
<td>1-mg DST serum cortisol, µg/dL</td>
<td>2.7 ± 3.6</td>
<td>1.7 ± 1.7</td>
<td>0.002*</td>
</tr>
<tr>
<td>Autonomous cortisol secretion#</td>
<td>42(32.6%)</td>
<td>32(22.1%)</td>
<td>0.051</td>
</tr>
<tr>
<td>Serum midnight cortisol level, µg/dL</td>
<td>8.2 ± 4.0</td>
<td>6.8 ± 2.3</td>
<td>0.035*</td>
</tr>
</tbody>
</table>

**Abbreviations:** ACTH, Adrenocorticotropic hormone; 1-mg DST, 1-mg overnight dexamethasone suppression test; eGFR, estimated glomerular filtration rate; iPTH, intact parathyroid hormone

*P < 0.05, ***P < 0.001
† Data after holding the medications that will interfere the renin-angiotensin-aldosterone system.

*A serum cortisol concentration > 1.8 μg/dL after an overnight 1-mg DST without any overt Cushing features confirms the diagnosis of autonomous cortisol secretion. [4]

As shown in Table 3, in logistic regression analysis, patients with PA concomitant ACS were associated with 24-h urine-free cortisol (P = 0.03), older age (P = 0.008), and diabetes mellitus (P = 0.025) (Fig. 3). The other associated clinical and biochemical factors for ACS patients were not significantly different.

### Table 3

<table>
<thead>
<tr>
<th>Odds Ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>1.04 (1.01–1.07)</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.60 (0.32–1.14)</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>1.00 (0.98–1.02)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>1.01 (0.97–1.05)</td>
</tr>
<tr>
<td>Plasma aldosterone concentration, ng/dL</td>
<td>1.00 (1.00-1.01)</td>
</tr>
<tr>
<td>Plasma renin activity, ng/mL/hr</td>
<td>0.98 (0.80–1.20)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.40 (1.12–5.12)</td>
</tr>
<tr>
<td>24-hr urine free cortisol ≥ 36 µg</td>
<td>1.91 (1.06–3.41)</td>
</tr>
</tbody>
</table>

**Abbreviations:** CI, confidence

*P < 0.05, ***P < 0.001

*A serum cortisol concentration > 1.8 μg/dL after an overnight 1-mg DST without any overt Cushing features confirms the diagnosis of autonomous cortisol secretion. [4]

**Discussion**

In this prospectively enrolled cohort, we found more than a quarter of PA patients concomitant with ACS. Our study suggested that 24-h UFC less than 36 µg was a cut-off point in exclusion of the patient that is possible ACS in patients with PA. Additionally, older age and diabetes mellitus were also risk factors for predicting ACS in PA patients. (supplementary Fig. 1)

Autonomous cortisol secretion, of which biochemical tests showed cortisol hypersecretion without clinical manifestations, had a high prevalence in PA patients. The presence of concomitant ACS in patients with primary aldosteronism (PA) was linked to more severe metabolic syndrome, as well as
severe left ventricular hypertrophy and poor renal function [2, 7, 8]. A previous study demonstrated a significant correlation between glucocorticoid excess and various metabolic risk factors, such as waist circumference, high-density lipoprotein, and diastolic blood pressure. Furthermore, the study showed that body mass index and insulin resistance, both of which are associated with obesity, were also affected by glucocorticoid excess. These findings suggest that the previously unidentified glucocorticoid excess in primary aldosteronism is a significant contributor to metabolic risk. [5]. Therefore, in such patients, it is important to figure out if there was excess cortisol secretion and 24-h UFC had shown to be useful to discriminate if there was excess cortisol secretion in the diagnosis of ACS [24].

The accuracy of overnight 1 mg DST can be influenced by the variable absorption and metabolism of dexamethasone. Factors such as concomitant use of drugs or alcohol, which can induce hepatic enzymes through CYP3A4, may lead to a reduction in dexamethasone levels. In addition, dexamethasone levels can vary significantly among healthy individuals who are not taking the drug. To mitigate the risk of false-positive or false-negative reactions, performing different types of examinations or repeating the measurement could help increase confidence in the test results.

24-h UFC provided a directed index of cortisol secretion. A previous study including Cushing's disease revealed that the 24-h UFC with 150 µg as a cut-off point in diagnosis of Cushing's disease had a sensitivity of 78.8% and a specificity of 52.5% [25], while in a previous meta-analytic study, the UFC was less sensitive compared to the other diagnostic tests in Cushing's syndrome with a sensitivity of 94.0% and a specificity of 93.0% [26]. In contrast to previous studies, our investigation comprised patients with primary aldosteronism (PA), while also excluding individuals who may have had hypercortisolism without Cushing's syndrome, such as those who were pregnant, had depression, alcohol dependence, or poorly controlled diabetes [27, 28]. Patients with Cushing's disease exhibited a higher midnight serum cortisol concentration compared to those without the disease. In our study, we also observed that patients with elevated urine-free cortisol levels had higher midnight cortisol levels, which could serve as an additional useful indicator of cortisol secretion.

A previous retrospective study identified a 24-hour urine-free cortisol (UFC) cut-off point of 70 µg for predicting adrenal cortical adenomas (ACS) with a sensitivity of 15.8%, specificity of 91.7%, positive predictive value (PPV) of 54.5%, and negative predictive value (NPV) of 63.4% [29]. However, this study also showed poor sensitivity in diagnosing ACS in the evaluation of adrenal incidentalomas. In contrast, our study demonstrated a higher NPV at a 24-hour UFC cut-off point of 36 µg, indicating that a daily urinary cortisol level of ≤ 36 µg could exclude the diagnosis of ACS in patients with PA. Our study also showed that diabetes mellitus and older age were also risk factors for predicting ACS in PA patients. The previous studies revealed a higher prevalence of diabetes mellitus in PA patients with ACS [30, 31] and a higher prevalence of ACS in older patients with adrenal incidentalomas [32–34]. In addition, about 15% of adrenal incidentalomas were associated with hormone secretion [35], which may also explain why age could be a risk factor for ACS, implying valuable insight into predicting ACS in PA patients.
Our study has some limitations and most of which are attributable to its data collection. The data from the Taiwan Primary Aldosteronism Investigation (TAIPAI) registry only recruited patients with a suspected PA diagnosis, which may cause selection bias. However, we included all consecutive patients who met the inclusion criteria of PA during the study period, ensuring comparable laboratory results. We also excluded patients who had known factors associated with false positive DST results, such as oral hormone contraceptive use, drug treatments that affect dexamethasone metabolism, liver disease, and psychiatric illness. However, the generalizability of our findings may be limited, as the database was constructed from medical centers and regional hospitals located in different cities in Taiwan. Factors such as income levels, race, ethnicity, and cultural practices in other countries may not be similar, which may affect the applicability of our results.

**Conclusions**

This study showed more than a quarter of PA patients concomitant with ACS, and a cut-off point of daily urinary cortisol level of fewer than 36 µg was useful for excluding ACS in patients with PA. Since ACS increases the risk of several chronic diseases and even cardiovascular events and mortality, it is important to develop definitive diagnostic methods for ACS in PA, and such studies should be further external validated in the future.

**Declarations**

**Conflict of interest**

All authors declare no conflict of interest.

**Ethics approval and consent to participate**

All experimental protocols research was approved by the Institute Research Ethical Committee of National Taiwan University Hospital (NTUH) (Http://doi.org/10.6084/m9.figshare.21730985).

All methods were carried out in accordance with relevant guidelines and regulations.

The informed consent was obtained from all patients or their legal guardian.

**Consent for publication**

Not Applicable

**Availability of data and materials**

The datasets generated and analyzed during the present study are available form the corresponding authors on request. The request of the new onset of DM was available by the principle of Taiwan National Health Insurance (NHI) program.
**Competing interests**

No

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**Authors’ contributions**

W.K.C and V.C.W. wrote the main manuscript text and prepared all figures. All authors reviewed the manuscript.

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Figures

Figure 1
depicted the generalized additive model (GAM) plot demonstrating the association between 24-hr urine free cortisol and the probability of autonomous cortisol secretion. The model incorporates the subject-specific (longitudinal) random effects expressed as the logarithm of the odds. The probability of autonomous cortisol secretion was constructed with age, sex, systolic blood pressure, diastolic blood pressure, plasma aldosterone concentration, and plasma renin activity. 24-hr urine free cortisol= 36 μg was a factor predicting autonomous cortisol secretion. The shaded areas indicate the 95% confidence intervals.

Figure 2

Sankey diagrams showing the 24-hr urine free cortisol in the primary aldosteronism patients with or without autonomous cortisol secretion.

Abbreviations: ACS, autonomous cortisol secretion; UFC, 24-hr urine free cortisol
Figure 3

summarized risk factors in predicting autonomous cortisol secretion in primary aldosteronism patients and revealed it were associated with higher 24-hr urine free cortisol (OR, 1.91 [95% CI, 1.06–3.41], P=0.03), older age (OR, 1.04 [95% CI, 1.01–1.07], P=0.008), and diabetes mellitus (OR, 2.4 [95% CI, 1.12–5.12], P=0.025).

Abbreviations: DBP, diastolic blood pressure; DM, diabetes mellitus; CI, confidence; OR, odds ratio; PAC, plasma aldosterone concentration; PRA, plasma renin activity, SBP, systolic blood pressure; UFC, 24-hr urine free cortisol $\geq 36 \mu g$

Supplementary Files

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