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Adrenocortical function in hypotensive patients with end stage renal disease ☆

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ABSTRACT

Background: Sustained hypotension among patients with end stage renal disease on dialysis (ESRDh) varies from 5.0% to 12.0%. Despite their role in the regulation of blood pressure (BP) corticoadrenal hormones have been poorly investigated.
Objectives: This study aims to detect adrenal insufficiency in ESRDh and follow their clinical outcome.
Methods: Fifty ESRDh and 30 healthy volunteers were studied. In all cases basal blood and saliva were obtained. Synthetic ACTH (25 μg) was injected intramuscularly and at 30 min saliva was collected. Circulating ACTH, renin, cortisol and aldosterone were measured and steroids were also assessed in saliva by immunoassay.
Results: Fifteen ESRDh achieved steroid responses not different than healthy volunteers; four had primary adrenal insufficiency; six had secondary adrenal insufficiency; nine had selective hypoaldosteronism and sixteen secondary hyperaldosteronism. The years on dialysis did not differ among subgroups. ROC analysis defined the following cut-offs for basal cortisol to predict adrenal insufficiency: in serum ⩽232.0 nM (sensitivity (S) 100.0% and specificity (E) 90.0%); in saliva ⩽4.4 nM (100.0% S and E). Basal aldosterone cut-off values to predict hyperaldosteronism were: in serum >500.0 pM and saliva >60.0 pM (100.0% S and E, for both). For the prediction of hypoaldosteronism the basal serum aldosterone was ⩽260.0 pM (100% S; 53% E) and in saliva it was ⩽20.1 pM (100% S; 58.5% E). Three patients with primary adrenal insufficiency and six with secondary adrenal insufficiency improved general clinical condition and normalized BP on steroids. One patient died before initiation of steroid therapy.
Conclusion: Adrenal function should be assessed in ESRDh in order to unmask adrenal insufficient states.

1. Introduction

Patients with ESRD may develop sustained arterial hypotension defined as systolic blood pressure levels <100 mmHg in the interdialytic period [1]. This is a critical clinical problem that affects the goal of dialysis that is to normalize the milieu interior as much as possible avoiding cardiovascular events. In most cases the pathophysiology of chronic hypotension remains unclear [1,2]. Several etiologies have been proposed: autonomic dysfunction, reduced cardiovascular response to cathecholamines and angiotensin II with associated decrease in receptor density, overproduction of vasodilator substances (nitric oxide, adrenomedullin) and cardiac dysfunction [2–4]. Yet, the available therapies directed to this sort

Abbreviations: ACTH, corticotropin; BMI, body mass index; BP, blood pressure; CAPD, continuous ambulatory peritoneal dialysis; E, specificity; ESRD, end stage renal disease; ESRDh, sustained hypotension among patients with end stage renal disease on dialysis; GC, glucocorticoid; HD, hemodialysis; HPA axis, hypothalamus–pituitary–adrenal axis; MR, mineralocorticoid receptor; S, sensitivity; SAF, salivary cortisol; SAL, salivary aldosterone.

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of hypotension have failed to report benefits. Physiologically, glucocorticoids (GC) modulate cardiac output and vascular smooth muscle tone by their permissive effects in potentiating vasoactive responses to vasoconstrictors (catecholamines, angiotensin II, vasopressin, endothelin and bradykinin) through glucocorticoid receptors [5,6]. In endothelial cells GC suppress the production of vasodilators such as nitric oxide and prostacyclin [7–9]. These substances and possibly other vasodilators were recently associated with chronic hypotension in a small group of patients on long term maintenance hemodialysis [4]. Aldosterone is the most important circulating mineralocorticoid and plays a major role in sodium and potassium metabolism by binding to mineralocorticoid receptors (MR) in kidney. These receptors are also responsible for extra renal actions of aldosterone. MR have been identified in heart, blood vessels and brain. Aldosterone modulates vascular tone and acts directly on the central nervous system increasing blood pressure in the setting of normal renal function [10–12]. These MR effects might persist along ESRD.

Despite their important role in the regulation of blood pressure glucocorticoid and mineralocorticoid hormones have not been deeply investigated as a main cause of chronic hypotension in patients with ESRD (ESRDh).

Different levels of circulating cortisol and ACTH concentrations were reported in patients with end stage renal disease (ESRD) [13–17]. Since 1974, studies on steroid adrenal response to ACTH stimulation were described as normal, subnormal and blunted in chronic renal failure [18–27]. In 1995 Harvey et al. [25] described the association of cortisol deficiency and hypotension in ESRD in two cases in whom substitution therapy with hydrocortisone and fludrocortisone normalized blood pressure levels. In 2006 our group was the first in exploring adrenal function through salivary steroids in a small series of ESRDh with normal albumin concentrations, diagnosing adrenal dysfunction in 6 out of 22 ESRDh [26].

Primary adrenal insufficiency is a rare disease [28] although its diagnosis is relatively easy in the general population. The challenge is to pose it in the context of ESRDh, as clinical findings of adrenal insufficiency are similar to those of renal failure. Anaemia and compromised peripheral vascular system impair blood sampling in ESRDh so a less invasive diagnostic approach through salivary steroids is recommended and should be available for these patients. In addition salivary cortisol and aldosterone have the advantage of reflecting the free circulating fraction ready to interact with target tissues [29]. The aims of the current study were: (1) To rule-out adrenal insufficiency through the assessment of salivary cortisol and salivary aldosterone in response to 25 μg of synthetic ACTH in a large series of patients on dialysis with sustained hypotension. (2) To study the involvement of the renin-aldosterone axis in ESRDh. (3) To assess the outcome of ESRDh patients with adrenal insufficiency on steroid replacement therapy.

2. Experimental

2.1. Study population

Fifty out of 71 chronic hypotensive patients with end stage renal disease (ESRDh) on dialysis replacement therapy were included in the study. They were referred to the Endocrine Unit of a University Hospital from two dialysis centres located within Buenos Aires metropolitan area. Forty-eight ESRDh (22 female and 26 men, aged 25–58 yo) were on hemodialysis 3 times a week (HD) and two (one female and one male, aged 39 and 65) on continuous ambulatory peritoneal dialysis (CAPD). They all had sustained hypotension (systolic blood pressure <100 mm/Hg for as long as 7.14 ± 2.15 months, registered 3 times a week before dialysis, requiring longer dialysis sessions in order to achieve adequate dry weight) and normal albumin levels. They had been on dialysis replacement therapy from 1 to 18 years (6.22 ± 4.74; median = 5.50). Their body mass index (BMI; mean ± SD) was 22.0 ± 1.9 kg/m^2. The etiologies of renal disease obtained by reviewing medical charts were: high blood pressure (17/50), diabetes mellitus type 2 (8/50), insterstitial nephritis (4/50), uremic hemolytic syndrome (3/50), kidney stones (4/50), proliferative glomerulonephritis (3/50), polycystic renal disease (3/50), systemic lupus erythematous (1/50), renal hypoplasia (1/50) and unknown (6/50). Cardiovascular disease was present (alone or combined) in 40/50 ESRDh. Left ventricular hypertrophy was prevalent (30/50) followed by peripheral vascular disease (15/50), coronary artery disease (13/50), cardiac arrhythmia (9/50) and valvular disease (4/50). Most patients were on drugs commonly used in ESRD (vitamins B, C, D, folic acid, phosphate and potassium binders and recombinant human erythropoietin). None ESRDh had had previous kidney transplantation or had been on mineralocorticoid receptor antagonists.

Control group included 30 healthy volunteers, 16 female and 14 male aged 20–58 yo with BMI 22.5 ± 1.5 kg/m^2. Their glomerular filtration rate ranged from 92.0 to 123.0 ml/min/1.73 m^2, without history of endocrine disease. All were normotensive and on sodium normal diet, females were evaluated in the early follicular phase.

Exclusion criteria were: infectious and psychiatric disease, amyloidosis, severe heart failure, intake of glucocorticoids (oral, inhaled or topical) and drugs that may affect steroideogenesis or ACTH secretion up to 6 months prior to the study, history of alcohol or substance abuse.

This protocol was approved by the Human Research Ethics Committee of the IDIM A Lanari, University of Buenos Aires, Argentina. All subjects gave written informed consent to participate in the study.

2.2. Study design

2.2.1. Salivary flow rate

All subjects rinsed their mouths with tap water to eliminate food contamination and avoided any oral intake and smoking at least 1 h before the test. Saliva was collected in pre-weighed polypropylene tubes and flow rate (ml/min) was calculated as described [26].

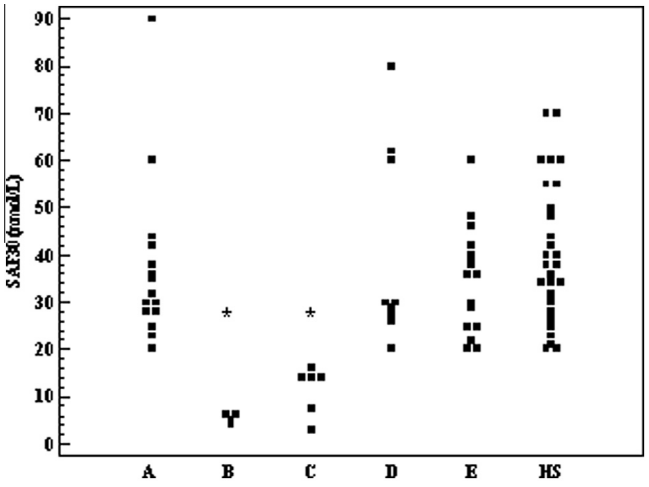

Fig. 1. Salivary cortisol (SAF_{30}) levels 30 min after 25 μg ACTH (i.m.) in hypotensive end stage renal disease patients (subgroups A–E) and healthy subjects (HS). Abbreviations: A, normal steroid responders to ACTH test; B, primary adrenal insufficiency; C, secondary adrenal insufficiency; D, selective hypoaldosteronism; E, hypereninemic hyperaldosteronism Statistical significance: $^*p < 0.05$ *vs.* HS and A.

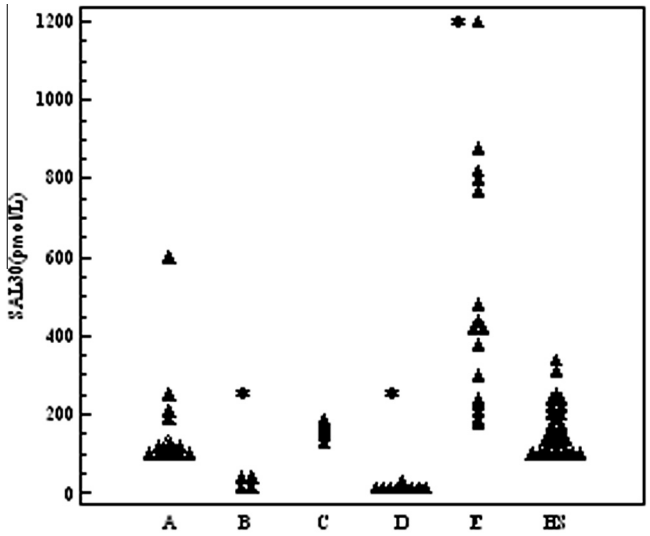

Fig. 2. Salivary aldosterone (SAL_{30}) levels 30 min after 25 μg ACTH (i.m.) in hypotensive end stage renal disease patients (subgroup A–E) and healthy subjects (HS). Abbreviations: A, normal steroid responders to ACTH test; B, primary adrenal insufficiency; C, secondary adrenal insufficiency; D, selective hypoaldosteronism; E, hypereninemic hyperaldosteronism. Statistical significance: $^{*}p < 0.05$ *vs.* HS and A.

2.2.2. Adrenocortical evaluation

After confirming the integrity of salivary gland function, the following protocol was performed by every ESRDh, simultaneously with healthy subjects, the day before the third HD session of the week or between two DPCA sessions.

Blood was drawn from 8.00 to 9.00 a.m. in seated position and subjects simultaneously collected 3.5 ml of whole saliva in sterile polypropylene tubes. A dose of 25.0 μg of synthetic human β^{1-24} ACTH (Synacthen; Novartis Pharma AG, Basel, Switzerland) prepared as previously described was directly injected into the deltoid muscle [29]. Salivary samples for cortisol and aldosterone were obtained 30 min after intramuscular ACTH stimulation. The supernatant obtained after centrifugation of saliva (1000*g*, 10 min) was kept at −20.0 °C until assayed. Basal plasma and serum samples were frozen at −20.0 °C until assayed. An extra serum aliquot was stored at −70.0 °C for 21-hydroxylase antibodies if necessary.

Salivary steroids were measured by RIA (coat-a count, Siemens Los Angeles, CA, USA) as previously described [29]. Salivary cortisol was expressed as nM and the minimal detectable salivary cortisol concentration was 0.5 nM. Salivary cortisol intra- and interassay coefficients of variation (CVs) were less than 6% and 13%, respectively. The cross-reactivities of the assay for prednisolone, 11-desoxycortisol, prednisone and dexamethasone were: 76.0%, 11.4%, 2.3% and 0.04%, respectively.

Salivary aldosterone was expressed as pM and the minimal detectable dose was 13.0 pM. The intra- and interassay CVs were less than 8.0% and 12.0%, respectively. The assay showed no cross-reactivity with cortisol and was negligible for other steroids.

Serum cortisol levels (nM) were assessed by RIA (coat-a count, Siemens Los Angeles, CA, USA). The minimal detectable dose was 6.0 nM. The intra and inter-assay CVs were less than 5.0% and 6.0%, respectively. Serum aldosterone levels (pM) were assessed by RIA (coat-a-count, Siemens Los Angeles, CA, USA). The detection limit for serum aldosterone assay was 33.0 pM. The intra and inter-assay CVs were less than 6.0% and 12.0%, respectively.

Plasma ACTH (pg/mL) was measured by IRMA (Diagnostic Systems Laboratories, Webster, Texas, USA). The detection limit was 1.3 pg/mL. The intra and inter-assay CVs were less than 9.4% and 8.0%, respectively.

Serum renin concentration (pM) was assayed by a two site immunoradiometric assay IRMA (Diagnostic Systems Laboratories, Webster, Texas, USA). The minimum detectable concentration was 0.06 pM. The intra- and interassay CVs were less than 3.0% and 4.0%, respectively. The aldosterone/renin ratio was calculated as aldosterone (pM)/renin (ng/L). To convert renin to ng/L, pM should be divided by 0.11 [30].

Serum anti-21 hydroxylase antibodies were measured by a radioligand assay (RSR limited, United Kingdom). The detection limit was 0.1 U/mL and intra and inter-assay CVs were less than 5.0% and 8.0%, respectively.

Blood analytes included in Table 1 were performed by the clinical chemistry laboratory for patients regular monitoring.

2.3. Statistical analysis

Results are expressed as mean ± SD and range. Data were analyzed by Mann–Whitney and Kruskal–Wallis tests. Correlations between serum and salivary steroid levels were evaluated by Spearman analysis. The ROC (receiver operating characteristic) curve was employed to graphically demonstrate the sensitivity and specificity of the different diagnostic tests (assessment of basal salivary and serum cortisol and basal salivary and serum aldosterone). In order to estimate the cut-off value, we used ROC analysis for the diagnostic setting which were optimized for sensitivity. Healthy subjects and ESRDh patients with normal steroid responses to ACTH were considered the "disease-free" group. Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 11.5, SPSS Inc, Chicago, USA). p values less than 0.05 were considered statistically significant.

3. Results

3.1. Salivary flow rate

Salivary flow rate in ESRDh (0.62 ± 0.23 ml/min; 0.20–1.10 ml/min) was not different than healthy subjects (0.65 ± 0.22 ml/min; 0.20–1.10 ml/min).

3.2. Correlation between basal steroids in saliva and serum

ESRDh had salivary cortisol (SAF) levels of 9.5 ± 5 nM (2.7–18 nM) and serum cortisol levels of 339 ± 103 nM (140–500 nM), correlating positively and significantly (r: 0.923, $p < 0.0001$) as in healthy subjects ($r = 0.91$, $p < 0.0001$). Basal salivary aldosterone (SAL) was 109 ± 158 pM (13.5–800 pM) and serum aldosterone levels 579 ± 708 pM (99–3800 pM), correlating positively and significantly (r: 0.90, $p < 0.0001$), as in healthy subjects ($r = 0.90$, $p < 0.0001$).

3.3. Clinical and biochemical characteristics of healthy subjects and ESRDh (Table 1)

ESRDh subgroups (A, B, C, D, E) were defined by the response of salivary steroids to ACTH stimulus. All ESRDh subgroups had potassium and PTH levels significantly higher than healthy subjects ($p \leqslant 0.0001$, for both) while hemoglobin and calcium concentrations were significantly lower ($p \leqslant 0.04$, for both). The years on dialysis therapy did not differ among ESRDh subgroups.

Healthy subjects achieved salivary cortisol $\geqslant$20.0 nM and salivary aldosterone $\geqslant$100.0 pM levels 30 min after ACTH stimulation (see Figs. 1 and 2). These salivary levels were in accordance with previous reported data [29].

Subgroup A (*normal responders*): 15 ESRDh had basal and stimulated SAF and SAL levels not different than those obtained in healthy subjects as well as circulating basal ACTH, cortisol and renin. These patients met the criteria of normal responders to ACTH

Table 1
Clinical and biochemical characteristics of healthy subjects and hypotensive patients with end stage renal disease subdivided into different groups (A–E) based on salivary cortisol and aldosterone response to ACTH stimulus.

Groups	ESRDh					Healthy subjects
	A	B	C	D	E	
Number	15.0	4.0	6.0	9.0	16.0	30.0
Age (years)	44.6 ± 8.1 (30.0–55.0)	51.0 ± 2.9 (47.0–54.0)	39.6 ± 8.0 (30.0–50.0)	41.0 ± 10.0 (25.0–54.0)	45.8 ± 9.4 (25.0–58.0)	43.7 ± 8.8 (25.0–58.0)
Female/male (ratio)	10.0/5.0	2.0/2.0	2.0/4.0	2.0/7.0	8.0/8.0	16.0/14.0
BMI (kg/m^2)	22.0 ± 1.3 (20.0–25.0)	20.8 ± 0.5* (20.0–22.0)	21.7 ± 1.25 (20.0–23.0)	22.2 ± 1.3 (20.0–24.0)	22.6 ± 0.92 (21.0–24.0)	22.5 ± 1.5 (20.5–25.0)
Dialysis (years)	7.0 ± 5.4 (1.0–17.0)	8.2 ± 3.9 (6.0–14.0)	5.8 ± 3.8 (3.0–13.0)	7.6 ± 6.3 (1.0–18.0)	4.3 ± 3.2 (1.0–9.0)	–
Serum						
Albumin (g/dl)	3.85 ± 0.35 (3.4–4.4)	3.87 ± 0.57 (3.3–4.5)	3.98 ± 0.35 (3.5–4.4)	3.93 ± 0.30 (3.5–4.4)	3.85 ± 0.29 (3.5–4.4)	3.92 ± 0.33 (3.3–4.5)
Na^+ (mEq/L)	139.8 ± 3.1 (135.0–145.0)	136.2 ± 1.5† (135.0–138.0)	136.8 ± 1.9† (135.0–140.0)	137.3 ± 2.9 (135.0–143.0)	138.6 ± 2.84 (135.0–144.0)	138.0 ± 2.7 (135.0–145.0)
K^+ (mEq/L)	4.8 ± 0.7* (3.8–6.0)	5.2 ± 0.5* (4.8–6.0)	5.0 ± 0.5* (4.2–5.5)	4.9 ± 0.9* (3.7–6.0)	4.6 ± 0.4* (3.8–5.5)	4.2 ± 0.2 (3.8–4.7)
Hb (g/dl)	11.1 ± 1.5* (8.5–14.0)	8.5 ± 0.4*† (8.0–9.0)	10.6 ± 0.5* (10.5–11.8)	11.5 ± 2.2* (8.5–14.5)	10.7 ± 1.5* (8.5–14.8)	13.9 ± 0.9 (12.5–15.6)
Calcium (mg/dl)	8.7 ± 0.2* (8.3–9.2)	8.5 ± 0.2* (8.5–9.0)	8.6 ± 0.1* (8.5–8.8)	8.7 ± 0.1* (8.5–9.5)	8.7 ± 0.2* (8.4–8.9)	9.2 ± 0.7 (8.5–10.5)
PTH (pg/ml)	96.6 ± 9.5* (85.0–120.0)	102.2 ± 6.1* (96.0–110.0)	102.2 ± 12.1* (85.0–120.0)	97.9 ± 8.6* (87.0–110.0)	102.6 ± 17.7* (79.0–142.0)	45.2 ± 19.9 (15.0–72.0)
ACTH (pg/ml)	25.7 ± 9.0 (12.0–42.0)	98.7 ± 16.9*† (85.0–123.0)	10.6 ± 3.7*† (5.0–15.0)	34.8 ± 8.9*† (20.0–48.0)	25.5 ± 10.5 (10.0–42.0)	22.0 ± 5.0 (15.0–30.0)
Renin (pmol/L)	1.4 ± 0.7 (0.3–3.0)	5.9 ± 5.8*† (0.11–14.0)	1.3 ± 1.0 (0.5–3.2)	0.2 ± 0.1*† (0.06–0.3)	6.9 ± 4.5*† (2.2–18.0)	1.8 ± 0.7 (1.0–3.1)
Cortisol (nmol/L)	364.3 ± 76.7 (250.0–500.0)	214.7 ± 17.07*† (200.0–230.0)	212.0 ± 18.5*† (189.0–232.0)	323.3 ± 105.2 (210.0–500.0)	389.3 ± 80.7 (290.0–500.0)	337.0 ± 100.0 (155.0–500.0)
Aldosterone (pmol/L)	245.8 ± 155.0 (99.0–500.0)	187.5 ± 79.0 (103.0–260.0)	308.8 ± 122.0 (240.0–500.0)	125.0 ± 60.8*† (100.0–260.0)	1302.2 ± 875.2*† (555.0–3800.0)	311.0 ± 161.0 (138.8–500.0)
Saliva						
Flow rate (ml/min)	0.64 ± 0.23 (0.2–1.1)	0.70 ± 0.27 (0.47–1.10)	0.62 ± 0.32 (0.2–1.1)	0.60 ± 0.26 (0.2–1.1)	0.61 ± 0.21 (0.2–1.0)	0.65 ± 0.22 (0.2–1.1)
ACTH test						
SAF_8 (nmol/L)	10.5 ± 3.7 (4.5–18.0)	4.0 ± 0.26*† (3.8–4.3)	3.7 ± 0.7*† (2.7–4.4)	9.8 ± 4.9 (4.5–18.0)	11.9 ± 5.2 (4.8–18.0)	11.0 ± 4.0 (4.5–18.0)
SAF_{30} (nmol/L)	37.0 ± 19‡ (20.0–90.0)	5.6 ± 1.3*† (4.2–7.0)	11.0 ± 5.0*†‡ (3.0–16.0)	40.0 ± 21.0‡ (20.0–80.0)	35.0 ± 11.0‡ (20.0–60.0)	39.7 ± 14.6‡ (20.0–70.0)
SAL_8 (pmol/L)	32.8 ± 18.8 (13.5–60.0)	13.6 ± 0.2 (13.5–14.0)	47.7 ± 13.6 (23.5–60.0)	14.5 ± 2.0*† (13.5–20.0)	272.0 ± 196.0*† (100.0–800.0)	34.0 ± 20.0 (13.5–60.0)
SAL_{30} (pmol/L)	165.0 ± 127.0‡ (100.0–600.0)	27.0 ± 15.0*† (13.5–40.0)	163.0 ± 22.0‡ (130.0–190.0)	16.0 ± 6.0*† (13.5–30.0)	497.0 ± 305.0*†‡ (180.0–1200.0)	165.0 ± 65.0‡ (100.0–340.0)

Abbreviations: ESRDh, hypotensive patients with end stage renal disease; A, normal steroid responders to ACTH test; B, subnormal SAF and SAL responders to ACTH stimulus; C, subnormal SAF and normal SAL responders to ACTH test; D, normal SAF and subnormal SAL responders to ACTH; E, normal SAF responders to ACTH stimulus with high baseline SAL concentrations; SAF_8, basal salivary cortisol; SAF_{30}, salivary cortisol 30 min after ACTH stimulation; SAL_8, basal salivary aldosterone; SAL_{30}, salivary aldosterone 30 min after ACTH stimulation.
To convert to SI units: albumin μmol/L = g/dl × 1.45; hemoglobin mmol/L = g/dl × 0.62; calcium mmol/L = mg/dl × 0.25; PTH pmol/L = pg/ml × 0.11; ACTH pmol/L = pg/ml × 0.22.
* Denotes significances compared with healthy subjects at $p < 0.05$.
† Denotes significances compared with group A at $p < 0.05$.
‡ Denotes significances compared with values after ACTH stimulation.

stimulus. They demonstrated a pituitary adrenal axis not different than healthy subjects, becoming an ESRDh reference group.

Subgroup B (*blunted SAF and SAL responders*): 4 ESRDh had basal salivary and serum cortisol levels significantly lower than healthy and A subjects. In contrast basal salivary and serum aldosterone did not show statistical differences. After ACTH stimulus both salivary cortisol and salivary aldosterone showed blunted responses in comparison with healthy and A subjects. Plasma ACTH and renin levels were significantly higher than in healthy and A subjects, except for one patient who had low renin levels. These patients had significantly lower BMI than healthy and lower sodium than A subjects.

Subgroup C (*subnormal SAF and normal SAL responders*): 6 ESRDh had lower basal salivary and serum cortisol than healthy and A subjects. Stimulated SAF concentrations were also lower than healthy and A subjects. At difference basal and stimulated SAL levels were not different than healthy and A subjects. In addition plasma ACTH levels were significantly lower than healthy and A subjects while renin did not show differences. Sodium was statistically lower than A subjects.

Subgroup D (*normal SAF and blunted SAL responders*): nine ESRDh had basal salivary and serum cortisol levels not different than healthy and A subjects and in response to ACTH stimulus. In contrast basal salivary and serum aldosterone showed lower concentrations than healthy and A subjects, with blunted aldosterone response. Plasma ACTH was significantly higher while renin was significantly lower than healthy and A subjects.

Subgroup E (*normal SAF responders with high basal and stimulated SAL concentrations*): sixteen ESRDh had basal salivary and serum cortisol concentrations not different than healthy and A subjects. Salivary cortisol response to ACTH was similar to healthy and A subjects. Baseline salivary and serum aldosterone were significantly elevated with higher salivary aldosterone concentrations in response to ACTH in comparison to healthy and A subjects. Plasma ACTH was similar to healthy and A subjects while plasma renin was significantly higher.

3.4. Endocrine diagnosis

Primary adrenal insufficiency was diagnosed in subgroup B. These patients complained of general weakness, weight loss, salt craving, chronic hypotension and hyperpigmentation (mild in 2 cases and severe in the other 2). Once the biochemical diagnosis was established, the following etiologies were disclosed: infectious (cytomegalovirus and tuberculosis) in two cases, autoimmune in one case (positive 21-hydroxylase antibodies) and unknown in the remaining patient.

Secondary adrenal insufficiency was diagnosed in subgroup C. These patients complained of lethargy, easy fatigability, anorexia, occasionally nausea and vomiting and chronic hypotension. Exploration of pituitary function was normal and sellar MRI did not show abnormalities. Insulin induced hypoglycemia was contraindicated in these patients and metyrapone was not commercially available in Argentina. A thorough anamnesis revealed that supraphysiological doses of glucocorticoids were administered during different emergency admissions up to six months before this study. Data obtained by reviewing charts revealed that cumulative prednisone dose varied from 20.0 to 25.0 g in a period of 12 months, suggesting the diagnosis of glucocorticoid related secondary adrenal insufficiency.

Selective hyporeninemic hypoaldosteronism was diagnosed in subgroup D. Seven patients had DM type 2 and two had past history of total nephrectomy for renal polycystosis.

Hyperreninemic hyperaldosteronism was diagnosed in subgroup E. The etiologies of renal disease were: polycystic renal disease ($n = 3$), hemolytic uremic syndrome ($n = 3$), renal hypoplasia ($n = 1$), kidney stones ($n = 2$), proliferative glomerulo-nephritis ($n = 1$), chronic arterial hypertension ($n = 3$) and unknown ($n = 3$). Plasma aldosterone/renin ratio was 22.0 ± 5.5, this ratio did not differ from healthy and A subjects (17.5 ± 7.5 and 19.5 ± 7.0, respectively), $p > 0.07$ for all.

3.5. Clinical outcome of adrenal insufficient ESRDh on steroid replacement

Three out of four patients with primary adrenal insufficiency (B) improved general clinical condition, gaining weight (BMI = 22.0 ± 0.8 kg/m^2), decreasing skin darkening and achieving sodium levels of 137.0 ± 2.6 mEq/L. Systolic blood pressure significantly increased from 69 ± 3 to 113 ± 3 mmHg ($p < 0.004$) on oral hydrocortisone (25–35 mg/day) and 9 alpha-fludrocortisone (0.1–0.3 mg/day) until present. The fourth patient with systolic blood pressure 62 ± 3 mmHg, died before steroid replacement therapy was initiated.

Six patients with secondary adrenal insufficiency (C) improved gastrointestinal symptoms and systolic blood pressure levels rose significantly from 90 ± 4 to 114 ± 4 mmHg ($p < 0.0001$) on hydrocortisone (20.0–40.0 mg/day) without changes in plasma sodium levels. Glucocorticoids were tapered along time until recovery of adrenal function (18–24 months). One patient died from liver malignancy.

All ESRDh with selective hypoaldosteronism (D) initiated therapy with 9 alpha-fludrocortisone. Only 4 patients followed this therapy along 2 months without improving blood pressure.

3.6. Accuracy of baseline salivary and serum steroids to predict adrenal dysfunction

Table 2 displays the accuracy of basal levels of both serum and salivary cortisol in diagnosing adrenal insufficiency, as well as the accuracy of both serum and salivary aldosterone in the diagnosis of hypo and hyperaldosteronism.

4. Discussion

Once the integrity of salivary acino-glandular function was ascertained, salivary steroids were studied in basal conditions and in response to low-dose intramuscular synthetic ACTH in order to assess dynamically the adrenal cortex. Basal salivary cortisol and aldosterone showed a positive and significant correlation with circulating homologous steroids ($r \geqslant 0.90$; $p = 0.0001$, for both).Primary adrenal insufficiency was diagnosed in 8% of the cases due to infectious, autoimmune and unknown disorders and secondary adrenal insufficiency in 12% due to exogenous steroid intake. Hypoaldosteronism with low renin was found in 18% of cases and secondary hyperaldosteronism in 32%. Adrenal secretory patterns were not related to dialysis therapy overtime. Blood pressure levels improved on steroid replacement therapy in patients with primary and secondary adrenal insufficiency. Full recovery of adrenal function was achieved in ESRDh with secondary adrenal insufficiency (subgroup C) after 24 months of glucocorticoid tapering.

Multiple oral disturbances in patients with ESRD have been described (e.g. dry mouth, uremic breath) most as consequence of a direct damage to the salivary glands probably associated with water restriction [31–33]. However in this study ESRDh showed an adequate salivary gland function in accordance with previous reports [26].

In ESRD the investigation of the adrenal reserve traditionally employed serum samples [20]. Recently salivary specimens have been used in dynamic testing as a less invasive clinical tool to assess adrenal function [27].

Table 2
Diagnostic performance of baseline serum and salivary steroids in hypotensive patients with end stage renal disease (ESRDh).

Diagnosis	ESRDh (subgroups)	Test (cut-off value)	Sensitivity [% (95% CI)]	Specificity [% (95% CI)]	AUC_{ROC} (95% CI)
Adrenal insufficiency	B, C	Serum cortisol (⩽232.0 nmol/L)	100.0 (69.0–100.0)	90.0 (80.5–95.9)	0.929 (0.848–0.974)
		Salivary cortisol (⩽4.4 nmol/L)	100.0 (69.0–100.0)	100 (94.8–100.0)	1.000 (0.954–1.000)
Hypoaldosteronism	D	Serum aldosterone (⩽260.0 pmol/L)	100.0 (75.1–100.0)	53.0 (38.5–67.1)	0.806 (0.688–0.894)
		Salivary aldosterone (⩽20.0 pmol/L)	100.0 (75.1–100.0)	58.5 (44.2–72.4)	0.807 (0.689–0.895)
Hyperaldosteronism	E	Serum aldosterone (>500.0 pmol/L)	100.0 (79.2–100.0)	100.0 (93.0–100.0)	1.000 (0.946–1.000)
		Salivary aldosterone (>60.0 pmol/L)	100.0 (79.2–100.0)	100.0 (93.0–100.0)	1.000 (0.946–1.000)

Serum and salivary samples were obtained in the morning (8.00 h). The cut-off values are estimated by ROC analysis and optimized for sensitivity.
Abbreviations: B, subnormal salivary cortisol (SAF) and salivary aldosterone (SAL) responders to ACTH; C, subnormal SAF and normal SAL responders to ACTH; D, normal SAF and subnormal SAL responders to ACTH; E, normal SAF responders to ACTH with high baseline SAL concentrations.

Despite the low prevalence of primary and secondary adrenal insufficiency described in the general population [34], a high number of patients with adrenal insufficiency were diagnosed through salivary steroids in ESRDh. Although arterial hypotension is not a main sign in subjects with secondary adrenal insufficiency it might be important in these patients exposed to stressing conditions [35]. The presence of hypotension in subgroup C suggests that dialysis is a stressing procedure that requires involvement of adrenocortical activity to maintain blood pressure levels. In addition, the recovery of H–P–A axis in this subgroup of patients took 18–24 months, reflecting the suppressive effect of steroids related to cumulative prednisone dose as described in renal transplant patients [36].

Hyporeninemic hypoaldosteronism diagnosed in ESRDh was ascribed to the involvement of the yuxtaglomerular system by diabetes mellitus (7/9) or bilateral nephrectomy (2/9), as reported [37]. In these patients ACTH levels were above the median (⩾20 pg/ml) and some had hyperkalemia, both endogenous inducers of aldosterone secretion. However, aldosterone remained blunted suggesting the presence of interfering factors on aldosterone secretion [38]. The administration of oral 9 alpha-fludrocortisone for a short period (2 months) in 4/9 of the cases did not improve blood pressure or potassium concentrations. This condition should be assessed in further studies in order to clarify if the failure of this treatment could be due to inadequate doses or to resistance to extra-renal actions of the drug.

ESRDh patients with hyperreninemic hyperaldosteronism revealed an overstimulation of the sympathetic and renin-aldosterone system [39–41] but a blunted vascular response to these effectors .

In order to find basal circulating and salivary steroid markers of adrenal status, ROC analysis showed that baseline salivary and serum cortisol (⩽4.4 and ⩽232.0 nM, respectively) were sensitive and specific enough to predict adrenal insufficiency as previously reported in a small series of ESRD (27). In contrast, basal aldosterone concentrations either in serum or saliva failed to accurately define hypoaldosteronism but showed high S and E to detect hyperaldosteronism at salivary and serum concentrations >60.0 and >500.0 pM, respectively. At present, some authors report different thresholds for circulating and salivary aldosterone to define states of overproduction of aldosterone, which may reflect the use of different assays [30,42,43].

This study has several limitations: autonomic dysfunction was partially investigated, vasodilators and catecholamines could not be assessed and patienťs consent for long term follow-up was not obtained.

It has been reported that ESRD patients on hemodialysis with normal or elevated blood pressure levels often exhibit a state of hypercortisolism [16,19] with elevation of late-night salivary cortisol and abnormal low-dose dexamethasone suppression [44,45]. When ESRD patients develop unexplained sustained hypotension unresponsive to regular therapies, affecting dialysis efficiency, adrenocortical function should be investigated. Salivary steroids offer a non-invasive alternative to easily assess adrenal function in ESRDh along their regular clinical follow-up.

Conflict of interest

The authors have nothing to disclose.

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