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Salivary Steroids in Response to ACTH

A Less Invasive Approach to Assess Adrenal Function in Hypotensive Patients With Chronic Renal Failure

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Abstract: The aim of this study was to use salivary cortisol and salivary aldosterone after an ACTH stimulation test to assess adrenal function in patients with chronic renal failure (CRF). Salivary samples for the measurement of cortisol and aldosterone were obtained before and after stimulation with 250 μg ACTH intramuscularly. Twenty patients with chronic renal failure and 24 healthy subjects were enrolled in the study. Salivary cortisol and salivary aldosterone concentrations were measured at baseline and 30 minutes after ACTH stimulation. Adrenal insufficiency was diagnosed in 5 patients. Selective hypoaldosteronism was found in 4 patients. The salivary steroid measurement after ACTH stimulation proves to be a useful test to diagnose adrenal insufficiency in patients with renal failure.

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Learning Objectives

- Compare the 20 patients in this study who had chronic renal failure (CRF) and sustained hypotension with a control group in regard to salivary flow rates and baseline serum and salivary levels of cortisol and aldosterone.
- Appraise the accuracy with which salivary steroid concentrations helped to identify primary and secondary adrenal defects and isolated hypoaldosteronism.

The ACTH stimulation test is used to diagnose adrenal insufficiency.[1,2] The simultaneous circulating assessment of cortisol and aldosterone in response to a standard dose of synthetic ACTH (250 μg) was first described by Dluhy et al.[3] This test has not been carefully characterized in patients with chronic renal failure.[4–14] Saliva sampling is a practical and less invasive method that allows stress-free multiple sampling in ambulatory patients. Cortisol and aldosterone concentrations in saliva appear to accurately assess adrenal activity, suggesting that salivary assays could be used to asses adrenal function.[15,16] The use of the simultaneous assessment of salivary cortisol (SAF) and salivary aldosterone (SAL) after ACTH stimulation for the differential diagnosis of adrenal insufficiency has been reconfirmed by our group.[17] At present, there are no reported data in the literature on the application of this dynamic test in patients with chronic renal failure (CRF) in whom adrenal dysfunction is suspected.

It is known that most patients with CRF have hypertension as a comorbid risk factor.[18] However, sustained hypotension, defined as systolic blood pressure less than 100 mm Hg, between dialysis sessions is a bad prognostic sign.[19–21] Treatment of symptomatic hypotension is difficult and many of the available therapies are marginally effective

and poorly tolerated.[22] Adrenal steroids are important in maintaining blood pressure, but their role in the pathophysiology of hypotension in patients on dialysis[19,23,24] is not well understood. Glucocorticoids (GCs) are required for normal cardiovascular reactivity to angiotensin II, epinephrine, and norepinephrine, thus contributing to the maintenance of cardiac contractility, vascular tone, and blood pressure. Glucocorticoids also decrease the production of nitric oxide, a major vasorelaxant and modulator of vascular permeability.[25,26] The renin–angiotensin–aldosterone system is also important in blood pressure regulation and volume homeostasis.[27] Aldosterone modulates vascular tone, although the exact mechanisms remain unclear. Aldosterone also has a direct action on the central nervous system to modulate blood pressure.[28,29]

This study was designed to assess adrenal function in patients with CRF whose main complaint was hypotension resistant to conventional therapy. The assessment of salivary steroids in response to ACTH stimulus could be particularly useful in these high-risk patients.

PATIENTS AND METHODS

The study included 20 patients with CRF (Table 1). All patients had sustained hypotension with blood pressure levels of 80.0 ± 6.0 mm Hg. Patients were not taking β-blocking agents, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers, diuretics, steroid therapy, or drugs that block adrenal steroidogenesis. Three patients (cases 3, 4, and 5) had received long-term steroid therapy (total prednisone dose 14.6 g, 4 g, and 6 g, respectively) and stopped it 12 weeks before this study. Hyperkalemia, defined as serum potassium ≥5.5 mEq/L, was found in patients 1, 2, 6, 7, and 20.

The control group included 24 normotensive healthy subjects (18 women and 6 men, aged 18–70 years). These subjects were taking no medication.

The study was approved by the Research Ethics Committee, School of Medicine, University of Buenos Aires, and all participants in the study gave written informed consent.

Study Protocol

All patients were studied twice with a 1-week interval between studies.

First Week

At 8.00 AM, a single fasting blood sample was drawn after 60 minutes of rest in the supine position with simultaneous collection of one sample of whole saliva. In patients on hemodialysis, this was performed before connection to the artificial kidney. Serum, plasma, and saliva supernatant were separated by centrifugation and stored at −20°C. Supine renin activity and plasma ACTH were measured in all control subjects and patients with abnormal responses to ACTH. Serum 21-hydroxylase autoantibody (21 OH Abs) levels were measured in all control

TABLE 1. Medical Background in 20 Patients With Chronic Renal Failure (CRF) and Sustained Hypotension

Patient	Age (y)	Sex	CRF Etiology	Dysautonomic Signs	Dialysis Modality	Time of Dialysis (years)	Cardiac Findings
1	47	M	HBP, RH	No	HD	6	No
2	56	F	Chronic pyelonephritis, total nephrectomy	Yes	HD	7	No
3	31	M	HBP, RH	No	HD	13	No
4	30	M	Interstitial nephritis	No	No	No	No
5	47	F	DM type1, Wegener	Yes	HD	2	No
6	31	M	HIV-positive	No	No	No	No
7	25	M	DM type1	Yes	No	No	No
8	43	M	HBP, hydronephrosis	Yes	HD	18	IC, LVH
9	42	M	Unknown	No	HD	2	No
10	48	F	HBP, proliferative glomerulonephritis	No	HD	7	No
11	51	F	HBP	No	HD	6	LVH, AF
12	25	M	Uremic hemolytic syndrome	No	HD	8	AF, LVH
13	36	M	Proliferative glomerulonephritis	Yes	HD	1	DC
14	74	M	HBP	Yes	CAPD	1	LVH
15	55	M	Unknown	Yes	HD	0.5	No
16	62	F	HBP	Yes	HD	6	LVH
17	41	F	Unknown	No	HD	2	No
18	54	F	HBP	No	HD	7	No
19	56	F	HBP, DM type 2	No	HD	1	No
20	54	F	HBP, interstitial nephritis	No	HD	15	PE

HBP indicates high blood pressure; DM, diabetes mellitus; RH, renal hypoplasia; PE, pericardial effusion; HD, hemodialysis 3 times per week; CAPD, continuous ambulatory peritoneal dialysis; LVH, left ventricular hypertrophy; AF, atrial fibrillation; DC, dilated cardiomyopathy; IC, ischemic cardiopathy.

subjects and in patients in whom blunted SAF and SAL responses to ACTH were demonstrated.

Second Week

Salivary Flow Rate. Patients rinsed their mouths with tap water to eliminate food contamination and avoided any oral intake and smoking at least 1 hour before the test. Between 7:00 and 8:00 AM, unstimulated whole saliva was obtained by spitting over a period of 5 minutes.[30] Before collection, the mouth was emptied by an initial swallow. At 30-second intervals, saliva was collected in preweighed polypropylene centrifuge tubes and flow rate (mL/min^{-1}) was calculated.[31]

After confirming that salivary flow rates were not different than those found in controls (see "Results"), the ACTH stimulation test was performed.

ACTH Stimulation Test. Whole saliva samples were obtained from fasting subjects at 9:00 AM after 60 minutes of rest in the supine position. Basal sampling was followed by the injection of 250 μg of synthetic ACTH (Synacthen; Novartis) into the deltoid muscle. Saliva samples were collected 30 minutes after ACTH stimulation. Saliva supernatant, obtained after centrifugation, was kept at −20°C for SAF and SAL measurements.

Salivary Steroid Measurements. SAF and SAL were measured by RIA (Diagnostic Products Corp., Los Angeles, CA) in saliva as previously described.[18] SAF was expressed as nmol/L and the minimal SAF concentration detected was 0.5 nmol/L. For SAF, intraassay and interassay coefficients of variation (CVs) were less than 6% and 13%, respectively. SAL was expressed as pmol/L and the minimal detectable dose of SAL was 13 pmol/L. The intra- and interassay CVs were less than 8% and 12%, respectively.

Blood Measurements. Serum cortisol levels (nmol/L) were assessed by RIA (Coat a Count; Diagnostic Products Corp.). The minimal detectable dose was 6.0 mmol/L. The intra- and interassay CVs were less than 5.0% and 6.0%, respectively. Serum aldosterone levels (pmol/L) were assessed by RIA (Diagnostic Products Corp.). The detection limit for serum aldosterone assay was 33.0 pmol/L. The intra- and interassay CVs were less than 6.0% and 12.0%, respectively.

Quantitative assessment of plasma renin activity (PRA, ng/mL/h) was measured by RIA of generated angiotensin I (Dia Sorin SRL, Italy). The limit of detection of PRA was 0.2 ng/mL/h. The intra- and interassay CVs were less than 9.9% and 11.5%, respectively. The serum aldosterone/PRA ratio was used as described by Olivieri et al.[32]

Plasma ACTH (pg/mL) was measured by IRMA (Diagnostic Systems Laboratories, Webster, TX). The detection limit was 1.3 pg/mL. Intra- and interassay CVs were less than 9.4% and 8%, respectively.

The levels of 21-OH antibodies (U/mL) were measured in serum using a radioligand assay (RSR Limited, U.K.). The detection limit was 0.1 U/mL. The intra- and interassay CVs were less than 5% and 8%, respectively.

Statistical Analysis

All results are given as mean ± standard deviation unless otherwise specified. The differences between baseline and stimulated steroid concentrations were computed using the nonparametric Wilcoxon test. The steroid levels before and after the ACTH stimulus were tested by the nonparametric Mann-Whitney *U* test. Correlations between serum and salivary samples were evaluated by Spearman analysis. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

Baseline Study

Salivary Flow Rate

The salivary flow rate in patients with CRF was not different from those in healthy subjects (0.66 ± 0.19 mL/min and 0.67 ± 0.22 mL/min, respectively, $P = 0.868$).

Serum and Salivary Steroids

Mean basal serum cortisol (332 ± 122 nmol/L) and aldosterone (580 ± 602 pmol/L) levels in patients with CRF were not different than control subjects (318 ± 80 nmol/L and 307 ± 99 pmol/L, respectively; $P \geq 0.689$). Basal SAF (9.4 ± 5.3 nmol/L) and SAL (73 ± 77 pmol/L) levels in patients with CRF did not differ from control subjects (9.3 ± 4.3 and 33.8 ± 15 pmol/L, respectively; $P \geq 0.153$).

SAF concentrations at baseline correlated significantly with baseline serum cortisol levels in patients with CRF ($r = 0.75$, $P = 0.0001$) and control subjects ($r = 0.79$, $P = 0.0001$) (Fig. 1A). A positive correlation was also found between basal SAL and serum aldosterone levels in patients with CRF ($r = 0.85$, $P = 0.0001$) as well as control subjects ($r = 0.78$, $P = 0.001$) (Fig. 1B).

ACTH Stimulation

Salivary Steroid Responses to ACTH

All control subjects achieved minimal SAF and SAL concentrations of at least 20 nmol/L and 100 pmol/L at 30 minutes after ACTH stimulation (Table 2). These values define the normal response.

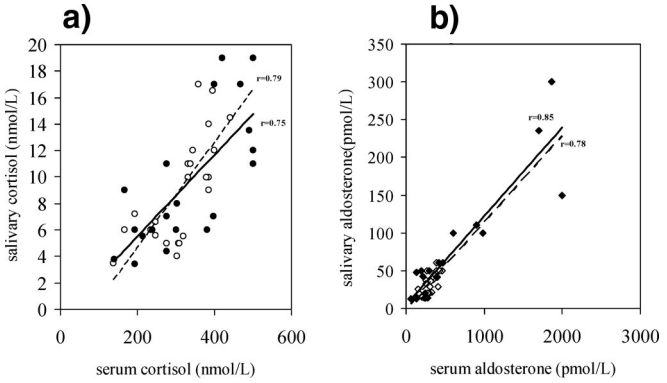

FIGURE 1. (A) Significant correlation ($P = 0.0001$) between cortisol levels in serum (●) and saliva (○) were observed in 20 patients with chronic renal failure (—) and in 24 healthy subjects (- - -). (B) Basal aldosterone concentrations in serum and saliva in patients with chronic renal failure (◆, —) and healthy subjects (◇, —) correlated significantly ($P \leq 0.001$).

TABLE 2. Individual Salivary Cortisol and Salivary Aldosterone Levels Before and 30 Min After ACTH Stimulation (250 μg intramuscularly) in 20 Hypotensive Patients With Chronic Renal Failure

	Baseline		After ACTH	
Patient	**Salivary Cortisol (nmol/L)**	**Salivary Aldosterone (pmol/L)**	**Salivary Cortisol (nmol/L)**	**Salivary Aldosterone (pmol/L)**
1	4	47	6.5	60
2	3.5	13	7	13
3	5.3	49	7.6	130
4	5.8	25	14	150
5	4.2	43	14	180
6	17	14	60	13.8
7	6.2	22	30	30
8	12.5	17	62	20
9	4.3	13	27	13
10	8	240	42	ND
11	11.5	106	22	ND
12	6.8	294	39	ND
13	13.2	108	38	ND
14	19.8	109	48	ND
15	4.7	158	21	ND
16	9.6	45	21	110
17	18.4	62	90	500
18	6.5	52	28	110
19	16.8	56	150	190
20	10.5	13.5	60	120
Control subjects				
Means ± standard deviation	9.3 ± 4.3	33.8 ± 15	38.2 ± 16.2	175 ± 83
Range	3.5–17	13.5–60	20–80	100–420

Control subject values are expressed as means ± standard deviation and range.
ND indicates not determined.

Patients 1 and 2 failed to increase salivary steroids 30 minutes after ACTH stimulus, suggesting a primary adrenal defect.

Patients 3, 4, and 5 showed a blunted SAF rise and a normal SAL response to ACTH stimulation suggesting a secondary adrenal defect.

In 4 patients (patients 6–9), SAF levels increased normally, whereas SAL concentrations did not change after ACTH stimulus. Selective hypoaldosteronism was suggested by these findings.

In patients 10–15, serum (1341 ± 583 pmol/L) and salivary (169 ± 80 pmol/L) aldosterone concentrations exceeded the upper limit of normal. SAF responses in these patients were similar to those obtained in control subjects. The etiology of the aldosterone excess was examined further.

Patients 16–20 showed normal SAF and SAL responses to ACTH.

Confirmation of Suspected Diagnosis

In patients 1 and 2, primary autoimmune adrenal insufficiency was diagnosed through the demonstration of ACTH (61.0 ± 1.5 pg/mL) and 21-OH Abs (5.0 ± 1.5 U/mL) levels above the upper normal range (5–40 pg/mL and 0.1–1.0 U/mL, respectively). High PRA (5.5 ng/mL/h) was found only in patient 1 (normal range, 0.5–2.0 ng/mL/h). Low PRA (0.1 ng/mL/h) was detected in patient 2 and was attributed to total nephrectomy (Table 1).

Secondary adrenal insufficiency was diagnosed in patients 3, 4, and 5 who had a history of long-term glucocorticoid therapy. These patients had normal ACTH levels (17 ± 3 pg/mL).

Isolated hypoaldosteronism was found in patients 6–9 in association with low PRA levels (0.25 ± 0.06 ng/mL/h).

Secondary hyperaldosteronism was confirmed in patients 10–15 with the detection of high PRA levels (13 ± 12.8 ng/mL/h; range, 4.4–37 ng/mL/h) and normal A/PRA ratio (6.0 ± 5.5; normal range, 11.7 ± 5.9).

DISCUSSION

This is the first study that evaluates adrenocortical function in patients with CRF with sustained hypotension assessed by measuring salivary steroids in response to ACTH stimulus. Salivary flow rate in patients with CRF was normal and a positive and significant correlation was found between salivary and serum steroids.[33,34]

The measurement of salivary steroids was shown to be a practical and less invasive approach to screening adrenal function in patients with CRF. We found that 9 of 20 patients had either primary or secondary adrenocortical insufficiency. The remaining 11 patients had normal adrenal reserve (5

cases) and aldosterone excess (6 cases). This latter condition was attributed to physiological adaptation mechanism to volume depletion.

We showed that in healthy subjects, 250 μg of ACTH stimulated serum and salivary cortisol levels of at least 552 nmol/L and 20 nmol/L, respectively. Simultaneously, aldosterone in serum and saliva reached levels of at least 555 pmol/L and 100 pmol/L, respectively.[35]

These results suggest that a baseline salivary sample followed by one salivary sample 30 minutes after stimulation with ACTH is capable of detecting either complete or selective adrenal insufficiency.

Primary adrenal insufficiency is an uncommon disorder whose clinical presentation includes the slow progression of malaise, fatigue, weakness, weight loss, anorexia, and hypotension. In patients 1 and 2 (Table 1), high ACTH levels confirmed the primary adrenal damage suggested by blunted salivary steroids responses to a rapid ACTH test. PRA levels were high in patient 1 and low in patient 2 as a result of total nephrectomy. The detection of high levels of 21 OH antibodies supported an autoimmune etiology[36] in both cases. Oral glucocorticoid and mineralocorticoid replacement in these patients was successful, resulting in improvement of well-being, correction of hyperkalemia, and normalization of systolic blood pressure levels to 120 mm Hg. Oral replacement with 9-α fludrocortisone in addition to hydrocortisone helped to balance K+ levels altered by the extrarenal effects of aldosterone described in uremia.[37] Recently, a clinical case report suggested the use of aldosterone replacement at physiological doses in a hyperkalemic patient with Addison disease undergoing hemodialysis, emphasizing the importance of aldosterone-driven secretion at extrarenal sites (colonic, salivary, and sweat glands).[38]

As expected, patients on long-term prednisone therapy had selective hypocortisolism. Tapering patients off steroids safely is a difficult problem for clinicians. To attain full recovery, several months on low steroid doses is necessary.[39] Only in patient 3 was there full recovery of adrenal function. Interestingly, 4 patients (patients 6–9) failed to increase aldosterone levels after ACTH stimulation, which has normal cortisol responsiveness. In these patients, the selective defect of aldosterone secretion coexisted with low basal renin activity levels, suggesting hyporeninemic hypoaldosteronism.[40] Although heparin may inhibit aldosterone secretion, this effect is not mediated by suppression of PRA levels.[41] Two patients (patients 6 and 7) had hyperkalemia ($K^+ \geq 6$), consistent with previous reports.[4,5,42] The primary illnesses in these 2 patients were diabetes mellitus and HIV, respectively. Both diseases are known to be associated with hyporeninemic hypoaldosteronism.[43,44] Only patient 8 agreed to replacement doses of 9-α fludrocortisone, resulting in improved blood pressure levels and overall well-being.

It is known that increased K^+ levels exert a direct effect on aldosterone secretion and that progressive CRF may cause chronic hyperaldosteronism.[45] Six patients with CRF (patients 10–15) had high salivary and serum aldosterone levels with normal K and elevated PRA concentrations. A primary adrenal aldosterone defect was ruled out in these patients by finding high PRA concentrations and a normal aldosterone/PRA ratio. Hyperreninemic hyperaldosteronism is common in patients on vasodilatory drugs or diuretics. Other causes include salt depletion, congestive heart failure, dehydration, emesis, and diarrhea. None of these were present in our patients. Then, aldosterone excess could be ascribed to the acute reduction in circulating plasma volume rather than to a derangement of the circulating components of the renin–angiotensin–aldosterone system as described.[46,47] In those cases in which hyperaldosteronism becomes a chronic feature, treatment with a competitive inhibitor of aldosterone can prevent the progression of ventricular hypertrophy.[48]

In 5 patients with CRF (patients 16–20), normal cortisol and aldosterone responses to conventional ACTH stimulation were observed. We conclude that salivary steroid measurements after ACTH stimulation can be an accurate and less invasive tool for the assessment of adrenal function in patients with CRF. Patients with CRF with sustained hypotension had a high incidence of adrenal hypofunction, either partial or complete. Improvement of blood pressure levels and life quality were observed in the follow up of patients receiving specific steroid replacement therapy.

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