0021-972X/99/$03.00/0
The Journal of Clinical Endocrinology & Metabolism

Vol. 84, No. 3
Printed in U.S.A.

Out-Patient Screening for Cushing's Syndrome: The Sensitivity of the Combination of Circadian Rhythm and Overnight Dexamethasone Suppression Salivary Cortisol Tests*

MARGARET CASTRO, PAULA C. L. ELIAS, ANA R. P. QUIDUTE, FERNANDA P. B. HALAH, AND AYRTON C. MOREIRA

Endocrinology Division, Department of Medicine and Department of Physiology, Faculty of Medicine, 14049–900 Ribeirao Preto, Sao Paulo, Brazil

ABSTRACT

Screening tests have been used to support a biochemical diagnosis of Cushing's syndrome (CS). Measurements of salivary cortisol offer facilities for studying out-patients. This study assessed salivary cortisol in screening for CS by evaluating hypercortisolism based on circadian rhythm and the overnight 1-mg dexamethasone (DEX) suppression test for out-patients. We evaluated 33 patients with CS. Thirty normal volunteers and 18 obese patients were used as controls. Salivary cortisol (nanograms per dL) levels (mean ± SEM) were 596 ± 44, 528 ± 104, and 1205 ± 118 (0900 h); 213 ± 27, 325 ± 76, and 778 ± 74 (1700 h); and 95 ± 8, 133 ± 26, and 914 ± 94 (2300 h) in normal controls, obese subjects, and CS patients, respectively. After the overnight 1-mg DEX test, they were 64 ± 1.1, 107 ± 25, and 1048 ± 129, respectively. In the present series, a single out-patient 0900, 1700, and 2300 h measurement and an overnight 1-mg DEX salivary cortisol level above the 90th percentile of the control or obese group values had sensitivities of 65.6%, 81.8%, 100%, and 100% or 78.1%, 57.6%, 93.3%, and 91.4%, respectively. The sensitivity improved (100%) in response to the combination of 2300 h and overnight 1-mg DEX salivary cortisol suppression tests to differentiate between obese and CS subjects. Our data indicate that nighttime sample and overnight 1-mg DEX suppression salivary cortisol tests are sensitive markers for the diagnosis of CS. In addition, the combination of the two tests improves the ability to differentiate between obese and CS patients and may be useful for out-patient screening. (*J Clin Endocrinol Metab* **84:** 878–882, 1999)

THE DIAGNOSIS and differential diagnosis of Cushing's syndrome (CS) have been among the most puzzling problems in endocrinology and remain controversial. Diagnostic difficulties can arise in distinguishing between CS and situations referred to as pseudo-Cushing states, such as severe obesity, high blood pressure, and depression (1–3), in which the clinical and biochemical evidence may be confusing. Therefore, the screening diagnosis applied to patients with a clinical suspicion of hypercortisolism is highly dependent on biochemical confirmation. The diagnostic strategies are usually based on measurement of urinary free cortisol (UFC), a low dose or overnight dexamethasone (DEX) suppression test, and evaluation of the diurnal variation of plasma cortisol (1–5). Recently, Yanovski *et al.* (6) reported the use of a DEX-CRH test to differentiate mild CS from normal subjects.

Twenty-four-hour urine collection for the measurement of UFC has been considered a gold standard for the diagnosis of CS (1, 2, 7, 8). This method has a sensitivity of 95% (5, 9) and usually provides a clear-cut distinction between patients with hypercortisolism and normal controls. However, in addition to the disadvantage of urine collection at out-patient and pediatric clinics (2, 5, 9, 10), in 4% of obese patients the cortisol production rate may be increased, leading to erroneous diagnosis of CS (9). The basis for the DEX suppression test lies in the deranged feedback relationship between ACTH and cortisol in CS. The diagnostic accuracy of the DEX suppression test may be compromised by incomplete 24-h urine collection, daily fluctuations in basal plasma cortisol, or failure to take the drug, mainly by out-patients submitted to a standard 2-day test (2, 3). Finally, although the morning plasma cortisol level may be normal in CS, the diurnal pattern of cortisol secretion is lost, as nocturnal levels are usually increased (11, 12). However, circadian rhythm is one of the most difficult tests of the hypothalamo-pituitary-adrenal axis to perform properly. The test requires hospitalization, the sample should not be obtained the first night in the hospital, the patient should not be told a blood sample will be collected, and its accuracy has been disappointing (3, 5).

In this context, measurement of cortisol in saliva may be helpful. Cortisol concentrations in saliva are independent of flow rate and transcortin fluctuations and reflect those in the free fraction of plasma (13, 14). In addition, salivary samples are obtained by noninvasive stress-free procedures, are easier to collect, even at home, and can obviate many problems such as situations where skilled personnel are not available (15). Salivary samples may be collected many times a day and may provide information of great diagnostic significance in the evaluation of CS (16). Recently, Papanicolaou *et al.* (17) and Raff *et al.* (18) reported that late night salivary cortisol

Received July 31, 1998. Revision received November 2, 1998. Accepted December 8, 1998.

Address all correspondence and requests for reprints to: Ayrton C. Moreira, M.D., Department of Medicine, Faculty of Medicine, 14049–900 Ribeirao Preto, Sao Paulo, Brazil.

* This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Grant 522219/96–9) and Hospital das Clínicas de Ribeirao Preto (HCFMRP-FAEPA).

determination is a sensitive marker (93% and 92%) for the diagnosis of CS in patients with mild hypercortisolism. Because of the continuing need for improved noninvasive means of distinguishing pseudo-Cushing from CS, the aim of this study was to assess salivary cortisol as a tool in screening for CS by evaluating hypercortisolism on the basis of circadian rhythm and the overnight 1-mg DEX suppression test for out-patients.

Subjects and Methods

Patients and normal subjects

We investigated in our department (between 1994–1997), after written informed consent was obtained, 30 normal nonobese healthy volunteers (13 men and 17 women; mean age ± SD, 28.3 ± 6.6 yr), 18 obese patients including some women with conditions resembling CS features such as essential hypertension and idiopathic hirsutism or menstrual disorders (4 men and 14 women; age, 23.1 ± 10.2 yr), and 33 patients with CS (2 men and 31 women; age, 25.5 ± 11.1 yr). The mean body mass index values were 23.5 ± 2.8, 39.5 ± 8.4, and 29.3 ± 6.4 kg/m^2, respectively, in the above groups.

Patients with both CS and obesity were referred to us with suspected clinical features of hypercortisolism. The diagnosis of CS was established by the lack of circadian variation in plasma cortisol levels and the lack of a fall in the plasma cortisol response to low dose (2 mg/day for 2 days) DEX administration. Of the 33 patients with CS, 20 were found to have Cushing's disease, 10 had cortisol-secreting adrenal tumors, 2 had primary pigmented nodular adrenocortical dysplasia, and 1 had adrenocortical nodular hyperplasia based on standard tests, including determination of plasma ACTH levels by RIA, high dose DEX suppression, and ovine CRH (oCRH) tests (19, 20). Plasma ACTH levels (mean ± SE) were 112 ± 26, 17 ± 3, 24 ± 0.9, and 11 pg/mL, respectively, in the above groups. In patients with pituitary-dependent CS, the percent suppression (mean ± SE) of plasma cortisol levels after the high dose DEX suppression test was 36 ± 8%, and there was an increase in plasma cortisol and ACTH after the oCRH test (20). In 13 patients with adrenal CS, all failed to suppress cortisol plasma levels in the high dose DEX test, and there was no increase in plasma cortisol or ACTH after oCRH treatment. Finally, the etiology of CS was confirmed by image studies, curative surgery, or positive tissue pathology.

Salivary cortisol assessment at 0900, 1700, and 2300 h

Saliva samples (1 mL) were collected in a plastic tube by direct spitting during a 15-min period at 0900, 1700, and 2300 h at the outpatient clinic or at the patient's home (21). The oral cavity was previously cleaned with tap water. The saliva samples were stored at 4 C until the following morning, when they were brought to the laboratory. After centrifugation at 2000 rpm, the transparent supernatants were stored at −20 C until assayed.

Overnight 1-mg DEX cortisol suppression test

One milligram of DEX was administered orally at 2300 h. The next morning, a post-DEX sample was taken for assay of salivary cortisol between 0800–0900 h.

Assays

Salivary cortisol measurements were made using a previously described RIA method in 25-μL samples of saliva without prior extraction or chromatography. This method previously demonstrated a good correlation (r = 0.95) with plasma free cortisol determined by equilibrium dialysis (22). The assay sensitivity was 62 ng/dL. The intra- and interassay coefficients of variation (CVs) were 5.5% and 11%, respectively. The assay showed parallelism between the standard curves and those obtained with different dilutions of saliva with high cortisol values. All samples obtained from each subject were analyzed in duplicate in the same assay.

Statistical analysis

A normal circadian pattern of salivary cortisol was defined as one in which both afternoon and nighttime salivary values were less than 83.5% of the morning level for each subject. This value was the result of the subtraction of 3 times the mean intraassay CV (16.5%) from the morning value, which was taken as 100% (15). We also used a second analysis of salivary cortisol fluctuations in circadian characterization by the method of Krieger *et al.* (23). In addition, for group analysis purposes, all individual data were combined. The mean afternoon and mean nighttime salivary cortisol values must be less than 67% of the mean morning value, which is taken as 100%. This percentage was the result of the subtraction of 3 times the interassay CV (33%).

The sensitivity and specificity for salivary cortisol were determined at different times and at various suppression levels. Levels of salivary cortisol above the 90th percentile of the normal control and obese patient groups were defined as cut-off points to calculate the sensitivity and the specificity of the test in distinguishing CS. These cut-off points cannot be considered absolute because they are defined by our dataset and may vary somewhat when applied to a new patient population.

Data are expressed as the mean ± SEM. Results that were below the detection limit of the assay were defined as the value of the detection limit. Friedman's ANOVA was performed for multiple comparisons. The Wilcoxon-Mann-Whitney test was used when appropriate. Significance was assumed when $P < 0.05$.

Results

Salivary cortisol levels: diurnal variation

Figure 1 shows individual salivary cortisol values obtained at 0900, 1700, and 2300 h and after the 1-mg DEX suppression test for normal controls, obese subjects, and CS patients. The mean (±SEM) values in each group are presented in Table 1. ANOVA demonstrated significant differences among the 0900, 1700, and 2300 h salivary cortisol levels for the normal control ($P = 0.0001$), obese ($P = 0.0002$), and CS ($P = 0.02$) groups. Circadian pattern of cortisol was completely characterized in the normal control and obese groups, but not in the CS group. Individual analysis showed that in contrast to 97% of the normal subjects and 73% of the obese subjects, 43% (13 of 30) CS patients had a persistence of a diurnal variation in salivary cortisol, but the levels were set abnormally high. The individual daily salivary cortisol

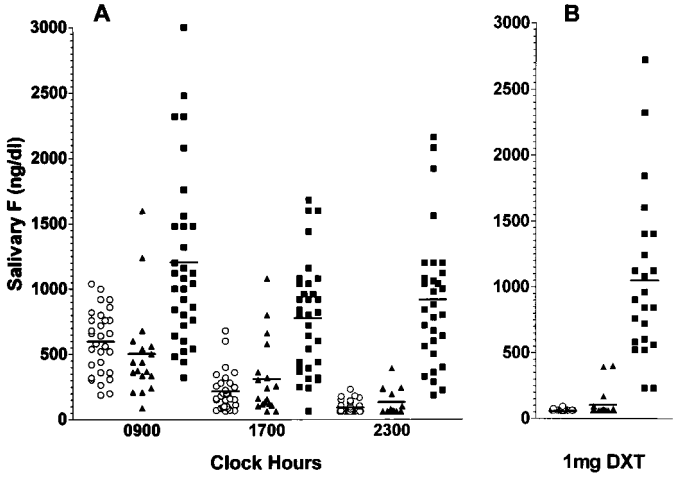

FIG. 1. Individual data points for salivary cortisol values obtained at 0900, 1700, and 2300 h (circadian rhythm; A) and after 1 mg DEX administered on the previous night (1 mg DEX; B) for 30 nonobese normal controls (○), 18 patients with obesity (▲), and 33 patients with CS (■). The mean is represented by the *horizontal bar*.

TABLE 1. Salivary cortisol levels at 0900, 1700, and 2300 h and after the overnight 1-mg dexamethasone suppression test (1 mg DXT) in normal subjects, obese patients, and Cushing's syndrome patients

Subjects	n	Salivary cortisol (ng/dL)							
		Mean ± SEM				Range			
		0900 h	1700 h	2300 h	1 mg DXT	0900 h	1700 h	2300 h	1 mg DXT
Control	30	596 ± 44	213 ± 27	95 ± 8	64 ± 1.1	188–1040	64–680	62–232	62–92
Obesity	18	528 ± 104	325 ± 76	133 ± 26	107 ± 25	88–1600	62–1080	62–392	62–400
Cushing's syndrome	33	1205 ± 118	778 ± 74	914 ± 94	1048 ± 129	320–3120	62–1680	184–2160	232–2720

Values are the mean ± SEM or the range.

TABLE 2. Sensitivity and specificity for salivary cortisol values at 0900, 1700, and 2300 h and after the overnight 1-mg dexamethasone suppression test (1-mg DXT) for the diagnosis of Cushing's syndrome compared as salivary cortisol levels above the 90th percentile of the normal control and obese groups

	Sensitivity (%)					Specificity (%)				
	0900 h	1700 h	2300 h	1 mg DXT	2300 h + 1 mg DXT	0900 h	1700 h	2300 h	1 mg DXT	2300 h + 1 mg DXT
Control *vs.* Cushing's	65.6 (21/32)	81.8 (27/33)	100 (30/30)	100 (23/23)	100 (23/23)	87.8 (29/33)	86.6 (26/30)	87.8 (29/33)	100 (28/28)	100 (28/28)
Obese *vs.* Cushing's	78.1 (25/32)	57.6 (19/33)	93.3 (28/30)	91.4 (21/23)	100 (22/22)	88.8 (16/18)	88.8 (16/18)	93.3 (14/15)	94.4 (17/18)	93.3 (14/15)

The number of subjects is in *parentheses*.

variation estimated by intraassay CV-based analysis was the same for all subjects, except two, compared to that obtained by the method of Krieger *et al.* (23). The salivary cortisol levels of patients with CS were significantly elevated at 0900, 1700, and 2300 h compared to those of obese patients ($P = 0.001$, $P = 0.002$, and $P = 0.001$, respectively) or normal subjects ($P = 0.0001$, $P = 0.0001$, and $P = 0.0001$, respectively). In contrast, the 0900, 1700, and 2300 h salivary cortisol levels of obese patients did not differ from those of normal volunteers. Although mean salivary cortisol levels were higher for CS patients, there was a marked overlap between groups at 0900 and 1700 h. The 2300 h salivary cortisol levels were undetectable in 60% of normal controls (18 of 30) and 38% of obese patients (7 of 18). The highest levels detected at this time were 232 and 392 ng/dL for normal controls and obese patients, respectively. The range of 2300 h values found in Cushing's syndrome was almost entirely outside the range of values found in the normal and obese groups. The overlap was due to 5 patients (15%) in the CS group.

Overnight 1-mg DEX suppression test

Salivary cortisol levels in CS were significantly elevated after the 1-mg DEX test compared to those in obese or normal groups ($P = 0.0001$). In contrast, there was no difference in salivary cortisol levels between obese and normal volunteers. In 28 of 30 control individuals, salivary cortisol was undetectable after the 1-mg DEX test, and the 2 normal subjects presented low detectable values (76 and 92 ng/dL). On the other hand, the post-1-mg DEX salivary cortisol level was detectable in every patient with CS. There was no overlap between normal and CS ranges. However, there was overlap between obesity and CS. Two of 18 obese patients exceeded a value of 232 ng/dL, the lowest found in the CS group.

Sensitivity and specificity

In the present series, a single out-patient 0900, 1700, and 2300 h measurement and overnight 1-mg DEX salivary cortisol levels above the 90th percentile of the control values had sensitivities and specificities of 65.6%/87.8%, 81.8%/86.6%, 100%/87.8%, and 100%/100%, respectively (Table 2). When we used salivary cortisol above the 90th percentile of the obese patients' values, the sensitivities and specificities were 78.1%/88.8%, 57.6%/88.8%, 93.3%/93.3%, and 91.4%/94.4%, respectively, at 0900, 1700, and 2300 h and after 1 mg DEX. The sensitivity improved (91.4% to 100%) in response to the combination of 2300 h and overnight 1-mg DEX salivary cortisol tests to differentiate between obese and CS patients.

Discussion

Measurements of salivary cortisol may have not modified the investigation of hospitalized patients, but offers facilities for studying out-patients. In our study, a comparison among the normal, obese, and CS groups revealed a substantial overlap of the basal 0900 h salivary cortisol levels. In this respect, our results were similar to those of other groups determining morning plasma free cortisol (24). Consequently, the usefulness of morning salivary cortisol is also quite limited and cannot be recommended as a test in the differential diagnosis of hypercortisolism.

The absence of a diurnal rhythm has been considered a hallmark of the diagnosis of CS (11, 12). However, circadian rhythm in patients with Cushing's disease has actually been demonstrated in some patients (25, 26). In this study, 43% of the CS patients presented diurnal variation of salivary cortisol assessed at 1700 and 2300 h, but the levels were detectable and abnormally high in all cases. Studies of spot or timed UFC between 2200–2300 h (7, 10) or a single sleeping midnight plasma cortisol measurement (27) have demonstrated that at 2300 h or midnight there was no overlap between normal subjects and CS. However, in these two studies, the control group was not ideal and would preferably have been formed from patients, particularly women with conditions resembling CS, such as obesity with essential hypertension and idiopathic hirsutism and polycystic ovarian syndrome.

Recently, it was demonstrated that a single serum cortisol value above 7.5 μg/dL at midnight discriminated CS from pseudo-CS patients with 93% sensitivity and 100% specificity (28). We reported salivary cortisol levels obtained at 1700 and 2300 h for 33 patients with CS compared to values in normal and obese groups. Whereas there was no difference in salivary cortisol evaluated at 1700 and 2300 h in the CS group, salivary cortisol was statistically higher at 1700 than at 2300 h in both the normal and obese groups. These data mean that the discrimination between CS and normal or obese groups improves in the late evening. In addition, the range of values found in CS patients at 2300 h was almost entirely outside the range of values found for the normal and obese groups. However, there was an overlap due to five patients with CS. A cut-off point for salivary cortisol above the 90th percentile of the normal subjects at 2300 h (168 ng/dL) produced a sensitivity of 100% and a specificity of 88%. This sensitivity is comparable to those reported in two recent studies using a single sleeping midnight plasma cortisol determination in patients hospitalized for a period of at least 48 h (27, 28). However, in routine practice, saliva can be obtained anywhere and at any time; therefore, this test is easily applicable at out-patient clinics. Our study further showed that the cut-off point of salivary cortisol above the 90th percentile (280 ng/dL) of the obese group at 2300 h produced a sensitivity of 93.3% and a specificity of 93.3%, demonstrating that the ideal control group should be patients with pseudo-CS states. This cut-off point of 280 ng/dL obtained from our data is similar to that recently reported (29), showing that the midnight salivary cortisol cut-off point of 270 ng/dL also detected 13 of 14 children with CS.

The overnight 1-mg DEX suppression test (30) is simple and reliable to segregate Cushingoid disorders from CS on an out-patient basis (1, 30). However, attempts have been made to improve diagnostic accuracy or simplicity (31). In the present study, we tested whether using salivary instead of plasma cortisol determination may simplify the 1-mg DEX suppression test. In fact, using as a criterion a cut-off point for salivary cortisol above the 90th percentile of the normal control (62 ng/dL) and obese (392 ng/dL) groups, the test had sensitivities and specificities of 100%/100% and 91.4%/94.4%, respectively. Based on our data, the overnight 1-mg DEX suppression test had a similar sensitivity and specificity as the 2300 h salivary cortisol determination.

We also evaluated the performance of the combination of both 2300 h collection and the overnight 1-mg DEX suppression salivary cortisol test to establish an easier way to differentiate CS from obese patients. Our data indicated that when the 2300 h collection and the overnight 1-mg DEX suppression salivary cortisol tests were combined, the sensitivity (100%) was higher than that of either test performed individually. Raff *et al.* (18) reported similar improvement in sensitivity combining an elevated late night salivary cortisol and an elevated 24-h UFC. The improvement in the sensitivity of the combined test may be a result of reducing laboratory error through an additional measurement. Flack *et al.* (8) estimated that at cut-off points for maximal sensitivity at 100%, specificity can vary by 4% only because of the daily variation in baseline cortisol secretion. In addition, our combined evaluation is based on two different abnormalities of the hypothalamo-pituitary-adrenal axis in CS: the altered circadian rhythm and the resistance to corticosteroid feedback.

Our data indicate that salivary cortisol determinations in a sample collected at bedtime (2300 h) and in the overnight 1-mg DEX suppression test are sensitive markers for the diagnosis of CS. In addition, the combination of the two tests improves the sensitivity to differentiate between obese and CS patients and may be useful for out-patient evaluation. Therefore, we recommend the association of both tests, because the collection of saliva rather than blood is a practical, simple, and noninvasive out-patient alternative to screen for hypercortisolism.

Acknowledgments

The authors thank Mr. J. Roberto Silva for technical assistance. We also thank Dr. George P. Chrousos for critical review of the manuscript, and Mrs. E. Greene for revision of the English text.

References

1. **Crapo L.** 1979 Cushing's syndrome: a review of diagnostic tests. Metabolism. 28:955–977.
2. **Bertagna X, Raux-Demay MC, Guilhaume B, Girard F, Luton JP.** 1995 Cushing's disease. In: Melmed S, ed. The pituitary, 1st ed. Cambridge: Blackwell; 478–545.
3. **Trainer PJ, Grossman A.** 1991 The diagnosis and differential diagnosis of Cushing's syndrome. Clin Endocrinol (Oxf). 34:317–330.
4. **Orth DN.** 1991 Differential diagnosis of Cushing's syndrome. N Engl J Med. 325:957–995.
5. **Findling JW, Doppman JL.** 1994 Biochemical and radiological diagnosis of Cushing's syndrome. Endocrinol Metab Clin North Am. 23:511–537.
6. **Yanovski JA, Cutler GB, Chrousos GP, Nieman LK.** 1998 The dexamethasone-suppressed corticotropin-releasing hormone stimulation test differentiates mild Cushing's disease from normal physiology. J Clin Endocrinol Metab. 83:348–352.
7. **Contreras LN, Hane S, Tyrrell JB.** 1986 Urinary cortisol in the assessment of pituitary-adrenal function. Utility of 24 hour and spot determinations. J Clin Endocrinol Metab. 62:965–969.
8. **Flack MR, Oldfield EH, Cutler GBJ, et al.** 1992 Urinary free cortisol in the high-dose dexamethasone suppression test for the differential diagnosis of the Cushing's syndrome. Ann Intern Med. 116:211–217.
9. **Mengden T, Hubmann P, Muller J, Greminger P, Vetter W.** 1992 Urinary free cortisol *vs.* 17-hydroxycorticosteroids: a comparative study of their diagnostic value in Cushing's syndrome. Clin Invest. 70:545–548.
10. **Laudat MH, Billaud L, Thomopoulos P, Vera O, Yllia A, Luton JP.** 1988 Evening urinary free cortisol: a screening test in Cushing's syndrome and incidentally discovered adrenal tumors. Acta Endocrinol (Copenh). 119:459–464.
11. **Boyar RM, Witkin M, Carruth A, Ramsey J.** 1979 Circadian cortisol secretory rhythms in Cushing's disease. J Clin Endocrinol Metab. 48:760–765.
12. **Liu JH, Kazer RR, Rasmussen DD.** 1987 Characterization of the twenty-four hour secretion patterns of adrenocorticotropin and cortisol in normal women and patients with Cushing's disease. J Clin Endocrinol Metab. 64:1027–1035.
13. **Walker RF, Riad-Fahmy D, Read GF.** 1978 Adrenal status assessed by direct radioimmunoassay of cortisol in whole saliva or parotid saliva. Clin Chem. 24:1460–1463.
14. **Umeda T, Hiramatsu R, Iwaoka T, Shimada T, Miura F, Sato T.** 1981 Use of saliva for monitoring unbound free cortisol levels in serum. Clin Chim Acta. 110:245–253.
15. **Santiago LB, Jorge SM, Moreira AC.** 1996 Longitudinal evaluation of the development of salivary cortisol circadian rhythm in infancy. Clin Endocrinol (Oxf). 44:157–161.
16. **Luthold WW, Marcondes JAM, Wajchenberg BL.** 1985 Salivary cortisol for the evaluation of Cushing's syndrome. Clin Chim Acta. 151:33–39.
17. **Papanicolaou DA, Mullen N, Nieman LK.** Diurnal salivary cortisol determination: an accurate and convenient test for the diagnosis of Cushing's syndrome. Proc of the 77th Annual Meet of The Endocrine Soc. 1995; 59.
18. **Raff H, Raff JL, Findling JW.** 1998 Late-night salivary cortisol as a screening test for Cushing's syndrome. J Clin Endocrinol Metab. 83:2681–2686.
19. **Moreira AC, Castro M, Machado HR.** 1993 Longitudinal evaluation of adrenocorticotrophin and β-lipotrophin plasma levels following bilateral adrenalectomy in patients with Cushing's disease. Clin Endocrinol (Oxf). 39:91–96.
20. **Borges MHS, Moreira AC, Kater CE.** 1995 The oCRH stimulation test in the

differential diagnosis of Cushing's syndrome. Arq Bras Endocrinol Metab. 39:44–51.
21. **Oliveira MHA, Moreira AC, Rossi A, Cruz C.** 1991 Salivary 17α-hydroxyprogesterone in management of congenital adrenal hyperplasia. J Pediatr Endocrinol. 4:255–262.
22. **Vieira JGH, Noguti KO, Hidal JT, Russo EMK, Maciel RMB.** 1984 Measurement of saliva cortisol as a method for the evaluation of free serum fraction. Arq Bras Endocrinol Metab. 28:8–10.
23. **Krieger DT, Allen A, Rizzo F, Krieger HP.** 1971 Characterisation of the normal temporal pattern of plasma corticosteroid levels. J Clin Endocrinol Metab. 32:266–284.
24. **Friedman TC, Yanovski JA.** 1995 Morning plasma free cortisol: inability to distinguish patients with mild Cushing syndrome from patients with pseudo-Cushing status. J Endocrinol Invest. 18:696–701.
25. **Refetoff S, Van Cauter E, Fang VS, Laderman C, Graybeal ML, Landau RL.** 1985 The effect of dexamethasone on the 24-hour profiles of adrenocorticotropin and cortisol in Cushing's syndrome. J Clin Endocrinol Metab. 60:527–535.
26. **Tourniaire J, Chalendar D, Rebbatu B.** 1986 The 24-hour cortisol secretory pattern in Cushing's syndrome. Acta Endocrinol (Copenh). 112:230–237.
27. **Newell-Price J, Trainer P, Perry L, Wass J, Grossman A, Besser M.** 1995 A single sleeping midnight cortisol has 100% sensitivity for the diagnosis of Cushing's syndrome. Clin Endocrinol (Oxf). 43:545–550.
28. **Papanicolaou DA, Yanovski JA, Cutler GBJ, Chrousos GP, Nieman LK.** 1998 A single midnight serum cortisol measurement distinguishes Cushing's syndrome from pseudo-Cushing states. J Clin Endocrinol Metab. 83:1163–1167.
29. **Gafni RI, Papanicolaou DA, Nieman LK.** Diurnal salivary cortisol measurement as a simple, non-invasive outpatient screening test for Cushing syndrome in children. Proc of the 80th Annual Meet of The Endocrine Soc. 1998; 169.
30. **Nugent CA, Nichols T, Tyler FH.** 1965 Diagnosis of Cushing's syndrome. Single dose dexamethasone suppression test. Arch Intern Med. 115:602–605.
31. **Laudat MH, Cerdas S, Fournier C, Guiban D, Guilhaume B, Luton JP.** 1988 Salivary cortisol measurement: a practical approach to assess pituitary-adrenal function. J Clin Endocrinol Metab. 66:343–348.

4TH International Workshop on Resistance To Thyroid Hormone

May 23–25, 1999
Reboucas Convention Center
São Paulo, Brazil

For further information contact: Geraldo Medeiros-Neto, MD, FACP, Thyroid Unit, Hospital das Clinicas, Av Eneas C Aguiar 155-8A Bl-3, 05403-900 São Paulo, Brazil. Phone +55-11-212-5711; Fax +55-11-211-5194; E:mail: medneto@uol.com.br