MARIA SILVIA SANTAREM CAETANO

AUMENTO DA PROBABILIDADE DIAGNÓSTICA DE SÍNDROME DE CUSHING SUBCLÍNICA EM UMA AMOSTRA POPULACIONAL DE PACIENTES ADULTOS OBESOS COM DIABETES MELLITUS TIPO 2

Tese apresentada à Universidade Federal de São Paulo - Escola Paulista de Medicina, para obtenção do Título de Mestre em Ciências pelo programa de pós-graduação em Endocrinologia

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Orientador: Claudio Elias Kater
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Dedicatória

Aos meus pais, que me ensinaram que o conhecimento é o mais sólido patrimônio que se pode possuir. Sua presença constante, amor, incentivo e apoio incondicionais sempre viabilizaram os projetos de minha vida. Meu amor, meu respeito e minha gratidão.

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De tudo na vida,
ficaram três coisas:
A certeza de que estamos sempre começando...
A certeza de que precisamos continuar...
A certeza de que seremos interrompidos antes de terminar...

Portanto, devemos:
Fazer da interrupção um caminho novo...
Da queda um passo de dança...
Do medo, uma escada...
Do sonho, uma ponte...

Fernando Pessoa

Sumário

Introdução

A Síndrome de Cushing (SC) é um complexo de sinais e sintomas que resulta da excessiva exposição dos tecidos ao cortisol. Harvey Cushing relacionou estes estigmas, compostos principalmente por obesidade, diabetes e hirsutismo, à presença de adenomas hipofisários basofílicos, definindo a doença que hoje leva seu nome [1]. Pouco depois, Walters e colaboradores identificaram a contribuição adrenal na etiologia dessa síndrome [2]. Na década de 70, Lewinsky descreveu os tumores adrenocorticais clinicamente silentes [3], e o conceito de síndrome pré-clínica ("Cushing subclínico") foi introduzido no início dos anos 80 por Charbonnel [4].

Na maioria das séries de hipercortisolismo endógeno, o adenoma hipofisário (Doença de Cushing) corresponde a aproximadamente 70% das causas de SC, enquanto os adenomas adrenais são responsáveis por cerca de 10% dos casos [5]. A distribuição por idade e sexo varia de acordo com a causa, sendo que a faixa etária de maior incidência ocorre entre os 25 e 50 anos [5,6]. Exceto pela secreção ectópica de ACTH, a SC é mais freqüente em mulheres do que em homens [5,7-9].

O diagnóstico laboratorial de hipercortisolismo endógeno constitui um desafio e as situações clínicas conhecidas como pseudo-Cushing, SC cíclica, hipercortisolismo leve e SC subclínica dificultam o diagnóstico [10].

A redução da capacidade do eixo hipotálamo-hipófise-adrenal (HHA) em responder ao *feed-back* negativo é o fundamento do teste de supressão com dexametasona (TSD). A partir do protocolo original descrito por Grant Liddle,

diversas doses e regimes de administração de dexametasona (DEX), assim como diferentes valores de normalidade têm sido utilizados [9,11-15]. As alterações do ritmo circadiano [9,16-19], têm sido um importante parâmetro para o diagnóstico da SC. A dosagem de cortisol em saliva, introduzida mais recentemente, e que pode ser colhida pelo próprio paciente em casa, facilitou a avaliação do ritmo de secreção do cortisol, sendo uma alternativa à dosagem no soro. No entanto, os valores de corte utilizados apresentam variações entre os diferentes estudos [20-23].

O excesso de produção de cortisol pode ser demonstrado através do cortisol livre urinário em 24 horas (preferencialmente em 3 amostras). Na investigação de SC, nenhum teste isolado pode confirmar o hipercortisolismo. Assim, a suspeita clínica deve ser confirmada pela combinação de testes funcionais diferentes [9,24].

Embora a SC seja considerada uma condição clínica rara, com uma incidência de 1 caso em cada 50.000-100.000 habitantes [5,25], a evolução das técnicas de imagem proporciona um número maior de diagnósticos de tumores adrenais. Essas evidências corroboram achados de necropsias que demonstraram presença de nódulos adrenais em 2-9% dos pacientes que não tinham sinais clínicos de disfunção adrenal [26,27].

A incidência de SC subclínica em incidentalomas adrenais, avaliada em diferentes séries, variou entre 5 e 20% dos pacientes [3,4,28]. A SC subclínica é caracterizada pela presença de resposta anormal a pelo menos dois testes de avaliação do eixo HHA, sem as manifestações clínicas típicas da SC [3].

O seguimento de pacientes com incidentalomas adrenais mostrou que a progressão da SC subclínica para a SC manifesta é questionável e, se ocorrer, rara [29] ; por isso, a maioria dos autores prefere o termo subclínico ao termo pré-Cushing.

O hipercortisolismo subclinico é um estado mais qualitativo do que quantitativo, e a ausência dos sinais clínicos característicos ocorre porque a magnitude da hipersecreção do cortisol é menor do que aquela vista na SC. As alterações no ritmo circadiano e nas provas de supressão são mais freqüentes do que o aumento dos níveis do cortisol urinário em 24 hs [4,26,28].

O excesso crônico de cortisol leva a características clínicas que são comuns com a síndrome metabólica (SM) [10].

Diferentes autores estudando pacientes com SC encontraram tolerância alterada à glicose, hipertensão arterial sistêmica (HAS) e obesidade em até 90%dos pacientes [30-33]. Essas características também são freqüentes entre pacientes com incidentaloma adrenal e SC subclínica [34,35]. O DM, a HAS e a SM são doenças de elevada prevalência na população mundial [36-39]. A alta mortalidade na SC está relacionada especialmente a eventos cardiovasculares [3,40], mas também ao tempo de exposição ao hipercortisolismo [41].

Pacientes com incidentaloma adrenal e SC subclínica que foram submetidos a tratamento cirúrgico apresentaram melhora do controle glicêmico e redução de peso e dos níveis pressóricos [3,4,35].

Leibowitz e colaboradores, avaliando indivíduos diabéticos dos tipos 1 e 2, mal controlados, encontraram SC em 3,3% dos pacientes avaliados [42]. Em outro

estudo, Catargi pesquisou SC oculta em pacientes com DM 2 mal controlado, em 200 indivíduos com índice de massa corporal (IMC) acima de 25 kg/m². O diagnóstico laboratorial e radiológico de SC foi confirmado em 5,5% e o anátomo-patológico em 2% dos indivíduos. Após o tratamento, os pacientes evoluíram com redução de peso e de hemoglobina glicada [43]. Esses achados sugerem que o hipercortisolismo silente não é completamente assintomático, enfatizando a importância de se realizar diagnóstico e tratamento, mesmo em pacientes com SCS.

O DM é uma doença de grande prevalência na população geral e ainda mais freqüente em pacientes com SC. A correção precoce de hipercortisolismo oculto reduz a morbidade e mortalidade a ele associadas.

O objetivo deste estudo prospectivo foi investigar a presença de SC em uma amostra populacional significativa de pacientes com DM2 e com sobrepeso ou obesidade, acompanhados no ambulatório de diabetes de nosso serviço.

Increased Diagnostic Probability of Subclinical Cushing's Syndrome in a Population Sample of Overweight Adult Patients with Type 2 Diabetes Mellitus

Aumento da Probabilidade Diagnóstica de Síndrome de Cushing Subclínica em Uma Amostra Populacional de Pacientes Adultos Obesos com Diabetes Mellitus Tipo 2

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Short title: *Cushing's Syndrome Among Overweight DM2*
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Abstract

Endogenous Cushing's syndrome (CS) is unusual. Patients with subclinical CS (SCS) present altered cortisol dynamics without obvious manifestations. CS occurs in 2-3% of obese poorly controlled diabetics. We studied 103 overweight adult outpatients with type 2 diabetes to examine for cortisol abnormalities and SCS. All collected salivary cortisol at 23:00h and salivary and serum cortisol after a 1mg dexamethasone suppression test (DST). Patients whose results were in the upper quintile for each test (253ng/dL, 47ng/dL and 1.8µg/dL, respectively for the 23:00h and post-DST saliva and serum cortisol) were re-investigated. Average values from the upper quintile group were 2.5-fold higher than in the remaining patients. After a confirmatory 2mgx2day DST the investigation for CS was ended for 61 patients with all normal tests and 33 with only one (false) positive test. All 8 patients who had two abnormal tests had subsequent normal 24h-urinary cortisol, and 3 of them were likely to have SCS (abnormal cortisol tests and positive imaging). However, a final diagnosis could not to be confirmed by surgery or pathology. Although not confirmatory, the results of this study suggest that the prevalence of SCS is considerably higher in populations at risk than in the general population.

Key words: Type 2 diabetes mellitus; Obesity; Overweight; Hypercortisolism; Cushing's syndrome; Subclinical Cushing's syndrome

Resumo

Aumento da Probabilidade Diagnóstica de Síndrome de Cushing Subclínica em Uma Amostra Populacional de Pacientes Adultos Obesos com Diabetes Mellitus Tipo 2.

A síndrome de Cushing (SC) endógena é rara. Pacientes com SC subclínica (SCS) apresentam hipercortisolismo sem manifestações clínicas. SC ocorre em 2-3% de diabéticos mal controlados. Estudamos 103 pacientes adultos obesos ambulatoriais com diabetes mellitus tipo 2 para avaliar alterações do cortisol e SCS. Todos coletaram cortisol salivar às 23:00h e cortisol salivar e sérico após teste de supressão com 1mg de dexametasona (DST). Pacientes cujos resultados de qualquer teste estavam no quintil superior (253ng/dL, 47ng/dL e 1,8µg/dL,
respectivamente para cortisol salivar 23:00h e salivar e sérico pós-DST) foram reavaliados. Os valores médios desse grupo encontravam-se 2,5 vezes acima dos valores dos demais pacientes. Após um teste confirmatório com 2mgx2dias DST a investigação da SC foi encerrada para 61 pacientes com todos os testes normais e 33 com apenas um teste (falso) positivo. Todos os 8 pacientes com dois testes alterados apresentaram cortisol urinário normal, mas 3 deles mostraram maior probabilidade diagnóstica de SCS (hipercortisolismo e alterações em exames de imagem). Contudo, o diagnóstico final não pode ser confirmado por cirurgia ou patologia em nenhum deles. Embora não confirmatórios, os resultados deste estudo sugerem que a prevalência de SCS seja maior em populações de risco do que na população geral.

Unitermos: Diabetes mellitus tipo 2; Obesidade; Sobrepeso; Hipercortisolismo; Síndrome de Cushing; Síndrome de Cushing Subclínica.

Introduction

The widespread use of potent synthetic glucocorticoids for a number of medical conditions is often associated with florid “cushingoid” manifestations. However, endogenous hypercortisolism or Cushing’s syndrome (CS) is considered an unusual disorder (1).

Endogenous CS is due either to primary (adrenal) or secondary (hypothalamic-pituitary) causes, the most common of which (80%) is “Cushing’s disease”, resulting from a corticotropin-secreting pituitary microadenoma. The cortisol-secreting adrenal adenoma, the second most common cause of CS, responds for less than 10% of the cases, although recently findings in reported series of adrenal incidentalomas point to a novel medical condition referred to as “subclinical CS” (3). In this setting, mildly altered cortisol dynamics is not associated with obvious clinical manifestations. In contrast, the metabolic syndrome resembles CS in several clinical aspects, but shares only a few abnormalities in cortisol dynamics.

The incidence of CS is estimated in 1:50,000 to 1:100,000 inhabitants of a general population (1,2). However, the metabolic syndrome (4) as well as the adrenal incidentaloma (2) has been reported increasingly worldwide.

Thus, it is conceivable that the incidence of CS could be higher than previously reported, provided screening tests are aimed to specific populations at risk. In fact, Leibowitz et al (5) and Catargi et al (6) found incidences of 2.2% and 2% in respectively 153 and 200 obese poorly-controlled diabetic patients.

Screening tests for the diagnosis of CS include: (1) late-night (23:00h) serum or saliva cortisol (to examine the absence of a circadian rhythm), (2) response to 1mg overnight dexamethasone suppression (to assess corticotroph resistance to the negative feedback), and (3) 24h-urinary free cortisol excretion rate (an indirect estimation of increased cortisol production) (6).

The former two tests are likely to be more sensitive in the detection of subclinical CS, in contrast to increased urinary free cortisol, that may be elevated only when clinical manifestations are present. Using appropriate cut-off values for greatest specificity, the sensitivity of these tests are generally acceptable to establish them as diagnostic standards for screening (7).

In CS, the frequency of obesity, glucose intolerance (with or without overt diabetes mellitus), and arterial hypertension reaches up to 90% of the patients (8-12). Nonetheless, each of these three features is highly prevalent worldwide (13-15). Although mortality is already elevated in obese, diabetic and/or hypertensive patients, it may be even higher in those with CS. When matched for age and gender, mortality of patients with SC was shown to be 4-fold higher than in the general population, mostly due to cardiovascular disease (16,17).

Medical and surgical cure or remission of the excess cortisol state in clinical or subclinical CS (adrenal incidentalomas) are associated with reduction in body mass and with significant improvement in glucose control and blood pressure levels (17,18).

In this paper we investigate adult overweight patients with type 2 diabetes mellitus (DM2) routinely followed in the Diabetes Clinic of our Institution, to verify the extent and magnitude of the abnormalities in cortisol dynamics and, as a consequence, if patients may have undiagnosed or occult CS, that could benefit from proper treatment.

Patients and Methods

We examined the medical charts of 285 sequential outpatients with DM2 who have been routinely followed in the Diabetes Center of the Division of Endocrinology at the Federal University of São Paulo, SP, Brazil. The diagnosis of DM2 has been previously established in all by current clinical, laboratory, and immunologic standards (19,20). Patients with the following criteria were excluded: (1) younger than 20 years of age, (2) BMI below 25kg/m^2, (3) pregnancy and breast-feeding, (4) previous or ongoing diagnosis of endogenous Cushing's syndrome, depression, chronic renal and/or hepatic insufficiency, and alcoholism, and (5) use of drugs that could potentially interfere with the diagnostic tests or laboratory assessment of cortisol, such as glucocorticoids, rifampicin, ketoconazole, carbamazepine, desmopressin, and mifepristone.

Of the 285 patient charts examined, 125 (44%) fulfilled the inclusion criteria and patients were summoned for a preliminary interview. One hundred and three of them (82.4%) - 69 female and 34 male, ranging from 36 to 82 years of age (median of 56) - agreed to participate in the protocol that had been previously approved by the Ethics Committee on Human Research from our Institution.

On a separate appointment, all 103 patients signed a written consent and were officially enrolled, having the following clinical and physical examination data compiled: time from

initial diagnosis of DM2 and hypertension, if present, weight and height, waist and hip circumferences (to calculate the waist:hip ratio – WHR), arterial blood pressure (BP), and medicines being used. Recent routine laboratory tests (within the last 30 days) were assembled, in special serum glucose, glicated haemoglobin (HbA1c), total and fractionated cholesterol, total blood count, creatinine, and urinalysis.

All patients were told to maintain their regular activities and routine medicines and to remain fast after 22:00h on a specified day. At 23:00h of that day they were instructed to collect saliva in a specific collector (Salivette®, Sarstedt, Germany) after oral hygiene with filtered water, and to keep the material under refrigeration until the next morning. Immediately after the saliva collection they should take 1mg dexamethasone (DEX, 2 x 0.5mg Decadron® tablets, Prodome, Brazil) with half a glass of water. The next morning all patients were seen in the laboratory where they had blood and a second saliva sample drawn between 08:00h and 09:00h.

Free cortisol was measured in the saliva and total serum cortisol, DEX and glucose in the blood samples. Although serum DEX was initially assessed to confirm ingestion and to validate the 1mg overnight DEX suppression test (DST), its levels were subsequently used for correlation purposes.

Instead of using pre-established test cut-off values for selecting patients at risk for CS, we chose to call back every patient whose test results were within the upper quintile (P80) for each test. This procedure introduces a higher sensitivity and would permit the investigation of a greater number of suspicious patients, in especial those who were in the “grey zone” for dynamic tests.

Additional Testing / Further investigation

Except for six patients (3M/3F) who subsequently declined additional testing, all other 23 who did not suppress saliva (values ≥47ng/dL) and/or serum cortisol (values ≥1.8 µg/dL) levels following a valid 1mg overnight DST (serum DEX >157 ng/dL at 08:00h, see below), and regardless of the 23:00h saliva cortisol level, were summoned for further investigation. An additional test was then performed, consisting of the classic low-dose DST (0.5 mg DEX PO every 6h for 8 doses) (21) in which blood samples were drawn before and 48h later for serum cortisol and DEX.

From this point on, and despite the exclusive response to the classic low-dose DST, patients who had two abnormal test responses (elevated 23:00h saliva cortisol and non-suppressible saliva or serum cortisol in response to either the 1mg overnight or the 2mg

DST) proceed to further investigation for CS, which included: (a) measurement of plasma ACTH, (b) dehydroepiandrosterone sulfate (DHEAS), (c) a 24h-urinary free cortisol, and (d) a DDAVP stimulation test (DDAVP®, 8 µg IV bolus, Ferring, Sweden), in which ACTH and cortisol were measured every 15 min for 2h (21,22). Finally, all patients had an adrenal imaging performed with fine-cut computerized tomography (CT) scans and a pituitary magnetic resonance imaging.

Figure 1 illustrates the algorithm used to investigate CS in this particular at-risk population sample.

Assays

Saliva material was centrifuged at 2,000 rpm and together with serum samples were kept frozen until the respective assays. Salivary cortisol was measured in 25 µl saliva aliquots by an in-house radioimmunoassay (RIA) without previous extraction or chromatography, as previously described (23). In brief, the intra- and interassay coefficients of variation were 4.4% and 5.1%, respectively, with a detection limit of 10 ng/dL.

Serum cortisol was measured by an in-house RIA (24,25). Serum DHEAS and plasma ACTH were also determined by commercially available chemiluminescent immunometric assay kits (Immulite® 2000 DPC, USA) and urinary cortisol by a RIA kit (DSL-2100 Active® cortisol, USA). Serum DEX was measured by an in-house RIA as follows: 50 µl of serum was added to rabbit anti-DEX antibodies (kindly provided by Dr. José Gilberto Vieira, Fleury Laboratory), and ^{3}H-DEX tracer (Amersham, USA), and then incubated at 4°C for 12-16h. The tracer-antibody reaction was interrupted, free antibodies were separated with dextran-charcoal and radioactivity was measured in the supernatant with a β-counter. Intra- and interassay CV are 6.3% and 6.0%, respectively, with the limit of sensitivity for that method set at 20 ng/dL.

For statistical purposes all values below the limit of sensitivity for the particular assay were arbitrarily considered equal to the detection value divided by the square root of 2 (26).

Screen Tests Cut-offs

The P80 cut-off or threshold values (that separate patients in the upper quintile) for each screening test were, respectively: 253 ng/dL, 47 ng/dL and 1.8 µg/dL, for the 23:00h and the post-1mg DST saliva, and the post-1mg serum cortisol. The same 1.8 µg/dL threshold value was used for the post-2mg DST.

Of note, these cut-offs values were close or similar to those previously reported to separate patients suspected to have CS from normal controls: 200 ng/dL (27) and 62 ng/dL (28), respectively for the late-night (23:00h) and post-1mg overnight DST saliva, and 1.8 µg/dL for the overnight 1 mg (22) as well as for the low-dose (2 mg x 2d) DST. Therefore, for the purposes of the present study we considered saliva and cortisol levels that were equal or higher than the P80 threshold as "positive test results" for hypercortisolism.
The overnight 1 mg and the 2 mgx2d DST were validated whenever 08:00h post-test serum DEX levels were >157 ng/dL (95% CL).

Statistical analysis

Comparison between variables was performed using the Mann-Whitney U test and Student's t test, where appropriate. Correlation coefficients were determined by the Spearman test. The level of statistical significance was set at 5% ($p<0.05$).

Results

Clinical Characteristics of the Population Sample (table 1)

Age, duration of DM2, BP, weight, BMI, WHR, and HbA1c from the 103 patients studied are presented in table 1, according to gender. Male and female patients did not differ regarding age, duration of DM2, BMI, and waist circumference, although weight and the WHR were significantly higher in men.
Most of the patients (81.5%, being 79.4% male and 82.6% female) were already hypertensive at the beginning of the study; even on treatment the average systolic and diastolic BP were still elevated. Duration of hypertension varied from 1 to 41 years (median of 10). HbA1c was significantly higher in women.

Hormonal Values (table 2)

Saliva (23:00h and post-1mg DST) and serum cortisol (post-1- and post-2mg DST) values are presented in table 2 as mean (±SD) and median (range), as well as the P80. As previously defined, values ≥ P80 (upper quintile) for each test were considered "suspicious" for diagnostic purposes, in contrast to the "normal" ones (below the P80 value). In the upper quintile group, 23:00h and post-1mg DST saliva cortisol values ranged from 253 to 527, and 47 to 117 ng/dL, respectively, whereas post-1mg DST

serum cortisol ranged from 1.8 to 7.8 µg/dL (figure 2). Average values from the upper quintile group were approximately 2.5- to 3-fold higher than in the remaining 80% of patients. When patients in the upper quintile group were compared to the remaining ones for any of the three tests, there were no significant differences regarding age, BMI, waist circumference, WHR, and HbA1c. However, systolic and diastolic BP were significantly higher in the upper quintile group.

Since DM has been occasionally associated to abnormalities in the HHA axis, we compared the results of saliva and serum cortisol to serum glucose or HbA1c levels and found no correlation with the test results.

Because the serum cortisol cut-off of 1.8 µg/dL used for the 1mg DST in the present study was similar to the value used systematically in the literature, we compared serum and saliva cortisol responses to 1mg DST using a 2X2 table: results were concordant in 84 patients (73 did suppress serum cortisol below 1.8 µg/dL and saliva cortisol below 47 ng/dL, whereas 11 did not), and were discordant in 18, nine “false-positives’ and nine “false-negatives” (figure 3).

Therefore, when the cut-off value of 47 ng/dL was used to define a positive response for the 1mg DST saliva cortisol, as compared to the 1.8 µg/dL serum cortisol, we obtained a sensitivity of 57.1% and a specificity of 89% for this test. Even with such a small sensitivity this value is already clearly below values used by others (28).

Validation of the DST was ascertained by measuring concurrently obtained serum DEX levels at 08:00h. Serum DEX levels ranged from 135 to 761 ng/dL (mean±SD of 346±122; median of 340). Using a cut-off value for “positive” DEX levels set at 157 ng/dL (95% CI), only one (out of 103) 1mg DST was considered invalid and therefore excluded from statistical analysis. The 2mgx2d DST was not validated (DEX < 157 ng/dL) in 3 of 4 patients who did not suppress serum cortisol levels; a normal and valid response was obtained in 19 (82.6%) patients.

Table 3. shows the clinical data of patients subgrouped according to test results: 61 patients (25%M / 75%F) had all 3 tests normal, 33 had only one (either a 23:00h saliva cortisol [n= 12] or a post-1mg DST saliva or serum cortisol [n= 21]) and eight had two abnormal test responses.

Patients with likely "Endogenous hypercortisolism"

Eight patients (4M/4F) had a tentative diagnosis of endogenous hypercortisolism made, due to a combination of elevated 23:00h saliva cortisol levels, and non-suppressible saliva or serum cortisol levels to 1mg and/or 2mg DST.

Individual clinical data and hormonal results for these eight patients are depicted in table 4. In all, a subsequent 24h-urinary free cortisol sample gave normal results, whereas basal plasma ACTH levels were subnormal (<10 pg/mL) in one (with serum DHEAS also subnormal), low-normal (10-20 pg/mL) in five (with serum DHEAS subnormal in one) and >20 pg/mL in two (both with normal DHEAS).

ACTH was unresponsive to a DDAVP stimulation test in six, four of whom had normal pituitary MR and adrenal CT imaging (table 4).

Four patients were diagnosed as having bilateral adrenal hyperplasia (BAH) on adrenal CT, one of whom with an additional 0.8 cm left adrenal nodule: one with an empty sella, one with a 5-6mm sellar nodule, and two with normal pituitary MR imaging.

The overall analysis of plasma ACTH, urinary free cortisol, serum DHEAS and DDAVP stimulation test, together with negative adrenal and pituitary imaging, made the probability of diagnosing CS in five of those eight patients rather slim.

The final three patients (# B, D, and H, in table 4) were likely to have subclinical CS, but in none this diagnosis could be surgically and/or pathologically confirmed so far. Patient B has bilateral adrenal hyperplasia (BAH) and an adrenal nodule, ACTH >20 pg/mL and a positive response to DDAVP, but has an unremarkable pituitary MR imaging. Although this may occur in up to 40% of patients with Cushing's disease (29,30), she declined inferior petrosal sinus sampling (IPSS) and has been followed clinically eversince.

Patient H has BAH with a 6mm pituitary nodule identified by MR. He also declined IPSS and subsequent surgery, if ever indicated.

Patient D is the most likely to present CS: she has BAH with an empty sella, repeatedly non-suppressible serum cortisol levels, but undetectable and DDAVP-unresponsive ACTH values. Partial investigation for macronodular adrenal hyperplasia resulted inconclusive, and a definite diagnosis could not yet be established.

Discussion

Among the classical manifestations of cortisol excess in CS, central obesity, glucose intolerance, and arterial hypertension predominate. These features are also characteristic of the metabolic syndrome, a highly prevalent condition among the general

population. The elevated mortality observed in patients with CS is not only associated to cardiovascular events (16,17), but also to the period of exposure to cortisol excess (31). Adult overweight type 2 diabetic patients, as in the case of the present study, also have a high prevalence of hypertension, being a distinctive risk group for the occurrence of CS.

The algorithm applied to investigate the extent and magnitude of cortisol abnormalities (figure 1) was a logical one in trying to screen and uncover patients who may have occult or undiagnosed CS. Using late-night saliva followed by the 1mg overnight DST, and measuring serum and saliva cortisol the next morning, we were able to exclude almost 60% of the patients in whom all results came back normal (below the P80 cut-off value for each test). By doing this we restricted the investigation to the most suspicious upper quintile subgroup, running only a minor risk of missing patients with CS but with false-negative results. In addition, because 23:00h saliva cortisol was considered the least robust among the screening procedures (28), we also excluded 12 additional patients in whom this was the only abnormal result, raising to 72% the total of patients initially excluded.

Except for post-DST saliva cortisol, the threshold values established in the present series - by choosing the P80 value for each test -, were close or even similar to those reported in the literature, that were most likely defined by ROC curves. Values for the 08:00h saliva cortisol after overnight DST have been previously reported by Castro et al (28), who defined different cut-off values according to the BMI: 62 and 392 ng/dL, respectively for non-obese and obese subjects. Although all of our patients were overweight by the inclusion criteria (BMI ≥25kg/m^2), the P80 cut-off value of 47 ng/dL for post-DST saliva cortisol was way below that proposed by Castro in the non-obese (28), presumably increasing test sensitivity.

Because the cut-off value for post-DST serum cortisol (considered our gold-standard test) was already highly sensitive (1.8 µg/dL), in accordance with that for the post-DST saliva cortisol (47 ng/dL), individual pairs of post-DST serum and saliva cortisol, which correlate positive and significantly, were concordant in 82.4% of the validated tests.

From the 29 out of the 103 original patients (28%), six declined further investigation. Also, from the remaining 23 patients (22%), 15 who had previously normal 23:00h saliva cortisol levels, had their investigation terminated since responded normally to a confirmatory 2mgx2d DST.

Thus, further detailed investigation was conducted only in the eight remaining patients who had at least two positive test results. When compared to patients who had normal responses to all tests and those with only one abnormal result, these eight patients did not disclose any particular clinical difference, except for being more hypertensive than the first group. Both serum glucose and HbA1c levels were similar, if not lower, than the other two groups. As expected for what is considered unsuspected or subclinical disease, only hormonal testing was abnormal (32-34).

Five of these eight patients were subsequently excluded due to normal ACTH, urinary free cortisol, serum DHEAS, and DDAVP stimulation test. Also adrenal and pituitary imaging were unremarkable. The final three patients (# B, D, and H, in table 4) were defined as subclinical CS. Because surgery or pharmacologic adrenalectomy was not attempted in any of them we were able to follow them critically for the past two years. In none, clinical manifestations of Cushing's syndrome ever became evident. In adrenal incidentalomas, progression of subclinical to overt CS is questionable, but probably occurs only occasionally (35).

Each patient from the original series who had only one abnormal test response was tentatively considered a false-positive for that test. In previous publications, functional abnormalities in the hypothalamic-pituitary-adrenal (HPA) axis were vaguely associated to the presence of obesity (36), hypertension (37) and diabetes mellitus (37,38). Most of these features were present in over 70% of our patients, and could easily explain some of the false-positive values, even in those who had two abnormal test results but in whom both adrenal and pituitary imaging were unrevealing.

However, this is still contradictory and open to discussion. We have previously demonstrated that obesity is not a cause of false-positive results for the 1mg overnight DST, although a cut-off level of 5 µg/dL was used at that time to define cortisol suppressibility (39). Others have found similar results (40). In the present study we also corroborate previous results (6) in that poor-controlled diabetes may not make patients more prone to biochemical abnormalities in the HPA axis. Possible correlations between HbA1c levels or the presence of hypertension with the responses to each of the tests used were lacking in our study.

In addition, we have attempted to exclude false-positive results in response to the DST by measuring serum dexamethasone levels. A serum concentration greater than 157 ng/dL was required for proper validation of the test. Similar results (220 ng/dL) were reported by others (41).

Absence of the circadian cortisol rhythm is generally considered the weakest among the screening tests for CS (42). This is due to the fact that several diseases and/or clinical conditions may be associated with a lack of cortisol decay towards the evening, in special depressive disorders (in whom the response to DST may also be positive), aging, and the presence of hypertension and/or diabetes (37). However, late-night salivary cortisol levels were reasonably sensitive and specific (92% and 93%, respectively) in discriminating patients with CS, from pseudo-CS, obese and non-obese control subjects (43). Also, in a recent review, Findling and Raff (44) suggest that 23:00h salivary cortisol, drawn in two different occasions, may be the best approach to screen patients for CS.

In reported series of patients with an adrenal incidentaloma and subclinical CS the absence of clinical manifestations is justified by normal cortisol production rates (35). This can be reflected by the normal 24h-urinary free cortisol levels observed in our patients.

Finally, taken altogether the results of the present study were very suggestive, although not confirmatory, that the prevalence of CS, in its subclinical form, is significantly higher in populations at risk for the disorder than in the general population, as previously observed by Leibowitz et al (5) and Catargi et al (6). Although current recommendations advise that only poorly-controlled diabetic patients should be investigated for subclinical CS, two out of our three most distinctive candidates for this diagnosis had reasonably controlled DM2.

It is also worth to mention that although subclinical CS is typical of patients with an adrenal incidentaloma, hypothalamic-pituitary disease may also be found incidentally, as possibly suggested in two of our patients.

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Figure legends:

Figure 1. Algorithm used in the present series to screen overweight adult patients with DM2 for Cushing's syndrome.

Figure 2. Saliva and serum cortisol levels in adult overweight DM2 patients evaluated for Cushing's syndrome. The shaded areas include values from patients who were in the upper quintile for each screening test (above the P80 thresholds that were respectively: 253 ng/dL, 47 ng/dL and 1.8 µg/dL, for the 23:00h and the post-1mg DST saliva, and the post-1mg DST serum cortisol). Values in the shaded areas were considered suspicious for endogenous hypercortisolism.

Figure 3. Correlation of concurrent serum and saliva cortisol levels after 1mg DST. Individual pairs of post-DST serum and saliva cortisol levels were concordant in 84 patients (placed in the upper right and the lower left quadrants).

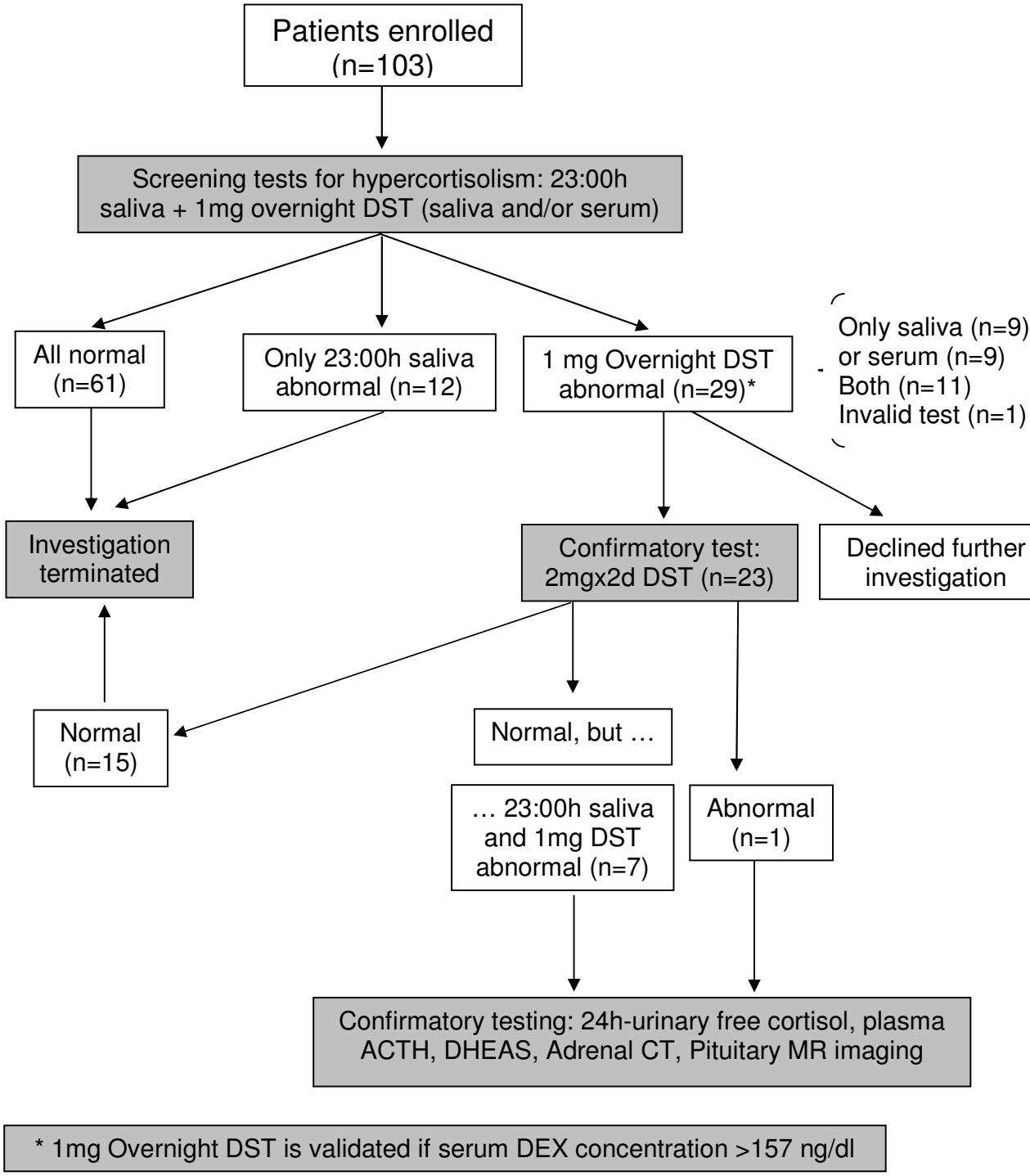
Patients enrolled (n=103)
Screening tests for hypercortisolism: 23:00h saliva + 1mg overnight DST (saliva and/or serum)
All normal (n=61)
Only 23:00h saliva abnormal (n=12)
1 mg Overnight DST abnormal (n=29)*
Only saliva (n=9)
or serum (n=9)
Both (n=11)
Invalid test (n=1)
Investigation terminated
Confirmatory test: 2mgx2d DST (n=23)
Declined further investigation
Normal (n=15)
Normal, but …
… 23:00h saliva and 1mg DST abnormal (n=7)
Abnormal (n=1)
Confirmatory testing: 24h-urinary free cortisol, plasma ACTH, DHEAS, Adrenal CT, Pituitary MR imaging
* 1mg Overnight DST is validated if serum DEX concentration >157 ng/dl

Figure 1.

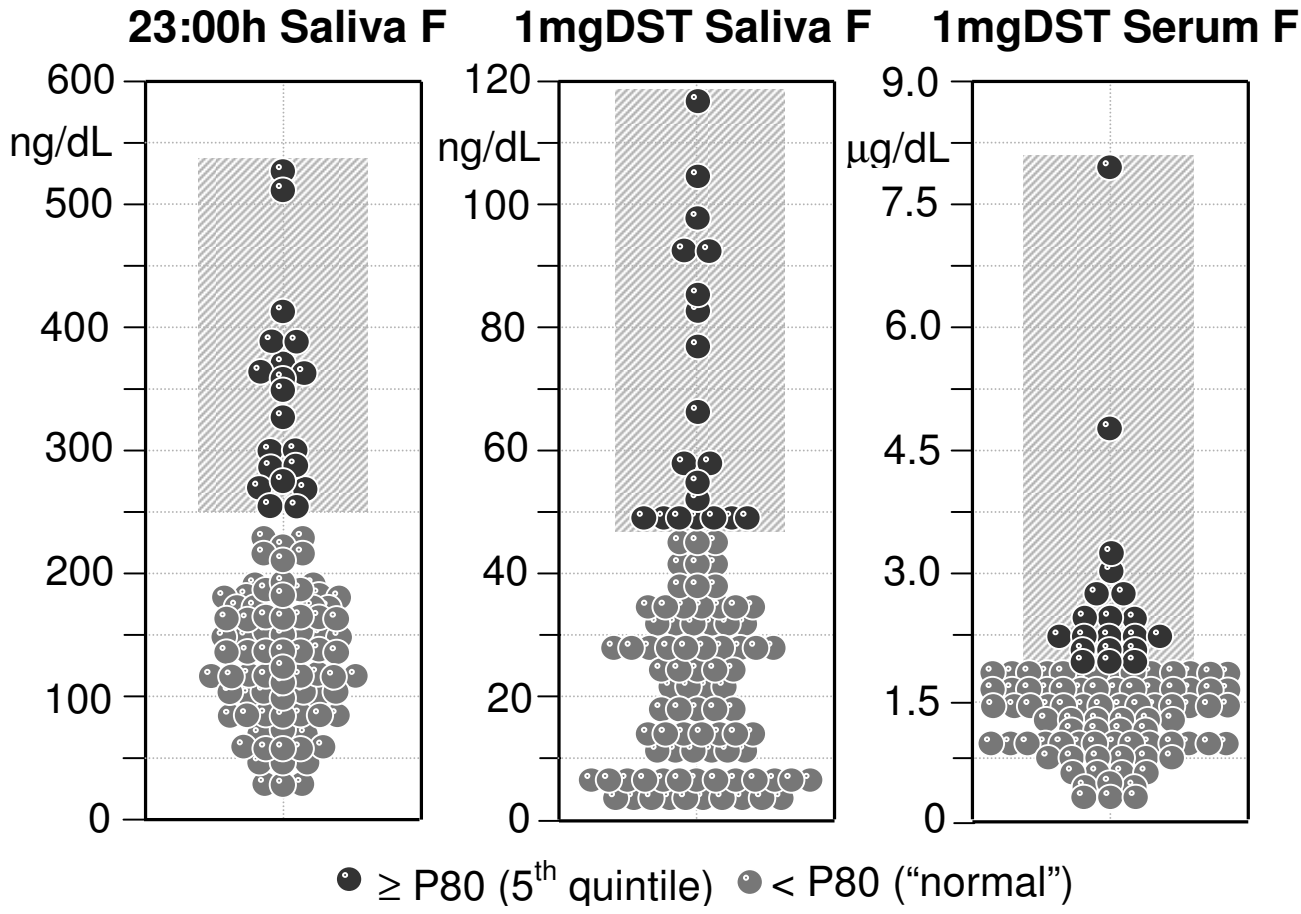
23:00h Saliva F
1mgDST Saliva F
1mgDST Serum F
600
ng/dL
500
400
300
200
100
0
120
ng/dL
100
80
60
40
20
0
9.0
µg/dL
7.5
6.0
4.5
3.0
1.5
0
≥ P80 (5th quintile)
< P80 ("normal")

Figure 2.

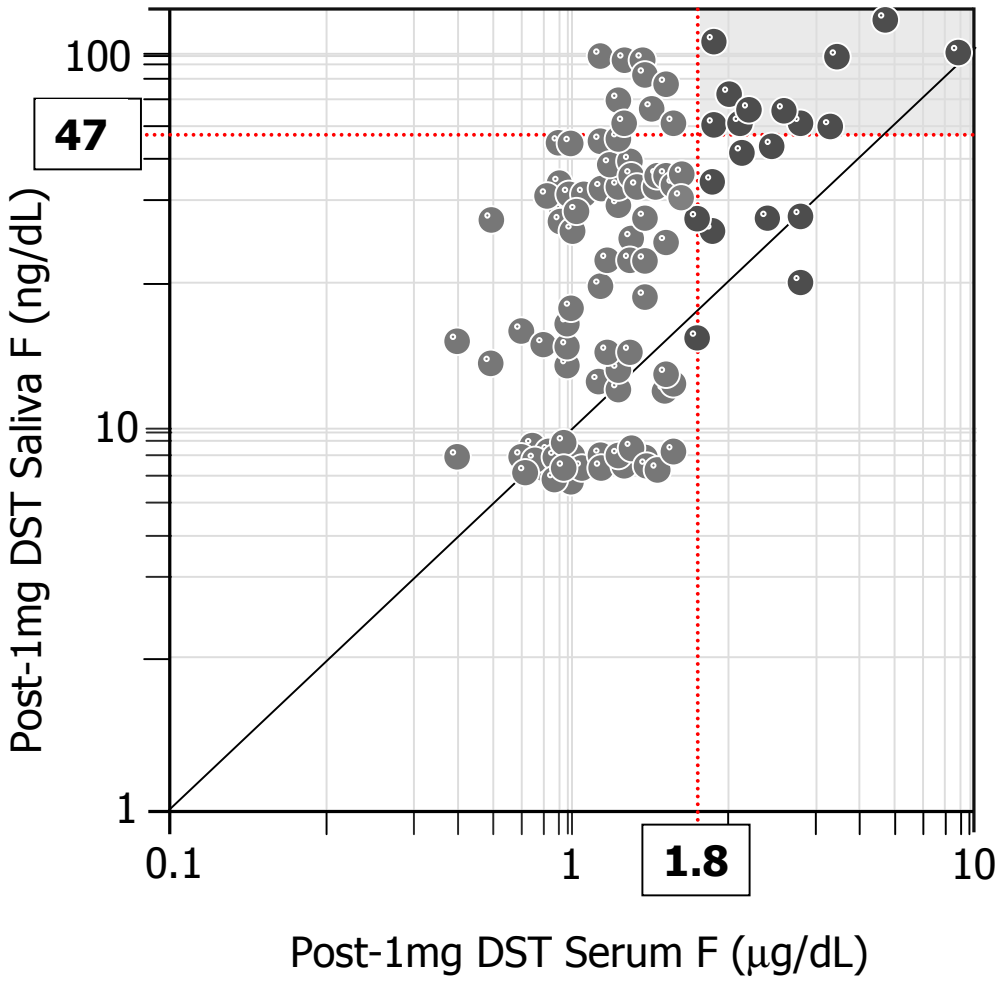
100
47
10
1
0.1
1
1.8
10
Post-1mg DST Saliva F (ng/dL)
Post-1mg DST Serum F (µg/dL)

Figure 3.

Table 1. Clinical characteristics of the 103 patients studied, according to gender.

		Total of patients (n=103; 34M/69F)	
		Mean±SD	**Median [range]**
Age (y)		56.4±8.8	56 [36-82]
Duration of DM (y)		10.8±7.5	9 [1-40]
BP (mmHg): Systolic		149.5±23.2	150 [110-220]
Diastolic		92.3±13.9	90 [70-130]
Weight (kg):	**M**	85.7±16.3	80.1 [65.9-132]
	F	75.7±12.2*	74.0 [51.5-110]
BMI (kg/m^2):	**M**	30.5±5.1	28.7 [25.0-43.6]
	F	31.7±4.7	31.4 [25.1-48.9]
Waist circumfer.:	**M**	102.4±12.0	99 [83-134]
(cm)	**F**	100.5±11.2	100 [72-136]
WHR:	**M**	0.99±0.05	0.99 [0.82-1.09]
	F	0.95±0.07**	0.95 [0.78-1.16]
HbA1c (%):	**M**	7.8±1.6	7.6 [5.3-12.0]
	F	9.0±2.3*	9.3 [5.1-15.6]

* P<0.01, M vs F (Mann-Whitney's test); ** P<0.005, M vs F (Student's t test)

Table 2. Mean (±SD), median (P50, and range) and P80 values of cortisol determinations in saliva (23:00hs and 08:00h after 1mg DST) and serum (after 1mg and 2 mg DST).

	Mean ±SD	**P50 or Median [range]**	**P80**
2300h Saliva (n= 103)	172.3 ± 101.9	154 [29.5-527.1]	253
≥P80	339.9 ± 77.6		
<P80	129.3 ± 49.4		
1mg Overnight DST			
Saliva (n= 102)*	30.4 ± 24.1	26.8 [7-117]	47
≥P80	69 ± 22.6		
<P80	21 ± 12.1		
Serum (n= 102)*	1.4 ± 0.9	1.2 [0.4-7.8]	1.8
≥P80	2.6 ± 1.4		
<P80	1.1 ± 0.3		
2mg x 2day DST (n= 20)#		0.9 [0.3-2.6]	1.8
≥P80 (n=1)	2.6		
<P80 (n=19)	0.8 ± 0.4		

*One and #Three false-positive tests were excluded (invalid)

Table 3. Clinical characteristics of the overweight diabetic patients grouped according to their responses to the functional tests for Cushing's syndrome: all normal (n= 61), one (n= 33) and two (n= 8) abnormal tests.

	All normal (n=61)		One abnormal test (n=33)				Two abnormal tests (n=8)	
			Salivary cortisol 23:00h (n=12)		Salivary or serum cortisol post-1mg DST (n=21)			
	Mean	Median	Mean	Median	Mean	Median	Mean	Median
Age (y)	55.8 ±8	56 [38-75]	54.3 ±9.4	53.5 [42-67]	59.5 ±8.8	58 [43-82]	53.3 ±10.3	55.5 [36-67]
Years of DM	10.7 ±7.7	9 [2-40]	11.8 ±7.6	9 [3-26]	11.0 ±6.2	11 [2-22]	10.1 ±10.1	7 [1-34]
Weight (kg)	78.1 ±14.2	75.8 [51.5-110.5]	78 ±13.6	75.6 [61.3-112.6]	81.9 ±15.4	79.8 [63.5-132]	82.1 ±15.2	76.9 [67.8-115.4]
BMI (kg/m^2)	31.3 ±4.9	30.3 [25-48.9]	31.4 ±4.9	30.3 [25.7-40.9]	31.7 ±4.9	30.8 [25.7-43.6]	31.2 ±4.4	30.9 [25.5-38.1]
Waist (cm)	100.9 ±11.2	101 [80-136]	102.9 ±7.6	101 [92-120]	101.5 ±13.0	99 [83-104]	102.5 ±10.8	99 [91-124]
WHR	0.96 ±0.07	0.96 [0.78-1.11]	0.97 ±0.05	0.98 [0.87-1.06]	0.96 ±0.08	0.98 [0.82-1.16]	0.97 ±0.06	0.98 [0.89-1.04]
SBP (mmHg)	145.9 ±21.5	140 [110-200]	142.5 ±20.9	135 [120-180]	160 ±22.8	160 [130-210]	160 ±32.1	155 [120-220]
DBP (mmHg)	89.7 ±11.2	90 [70-110]	88.3 ±15.3	90 [70-110]	100.5 ±16	100 [80-130]	96.3 ±18.5	100 [70-120]
HbA1c (%)	8.7 ±2.3	8.4 [5.1-15.6]	8.3 ±1.5	8.8 [5.3-9.9]	8.8 ±1.8	9.4 [6.0-12.5]	7.8 ±2.7	6.9 [6.2-14.4]
Glicemia (mg/dL)	151.2 ±74	132 [53-488]	135.5 ±49.7	122 [58-223]	158 ±56.9	144 [93-365]	134.5 ±40.7	128 [74-192]
23:00h Saliva F (ng/dL)	122.5 ±46.5	117 [30-212.1]	325.9 ±76.7	299.4 [253-512]	149.2 ±53.4	156 [29.5-232.7]	367.3 ±79.9	370.5 [264-527.1]
1mg-DST Saliva (ng/dL)	20.2 ±11.8	17.5 [7-46]	18.5 ±14	13 [7-46]	54.1 ±23.9	49 [16-105]	63.8 ±34	58.3 [20.1-117]
1mg-DST Serum (µg/dL)	1.1 ±0.3	1.1 [0.4-1.7]	1.1 ±0.4	1.2 [0.4-1.7]	1.9 ±0.6	1.9 [1.1-3.2]	3.1 ±2.2	2.4 [1.3-7.8]
DEX (ng/dL)	348.2 ±128.5	339 [140-761]	333.4 ±135.2	345 [159-660]	359.7 ±96	356 [165-514]	311.4 ±126.9	314 [135-476]

Table 4. Clinical and laboratory data of eight patients with likely "endogenous hypercortisolism", investigated for Cushing's syndrome.

								Cortisol									
ID	**Sex/ age**	**Years of DM**	**BMI**	**Waist**	**WHR**	**↑ BP**	**HbA1c**	**Saliva 23:00**	**Saliva 1mg**	**Serum 1mg**	**Serum 2mg**	**ACTH**	**DHEAS**	**FU**	**DDAVP**	**Adrenal CT**	**Pituitar MR**
A	F 36	5	32.3	98	1.04	Yes	7.0	**264**	20.1	**2.7**	*	12.0	162	65	-	Nl	Nl
B	F 43	11	29.6	94	0.92	Yes	14.4	**371**	**48.7**	**2.1**	0.9	28.3	97	50	**+**	**BAH+Nodule**	Nl
C	F 56	6	33.1	100	1.00	Yes	7.8	**283**	27	**2.7**	1	13.7	40	73	-	Nl	Nl
D	F 67	34	36.0	113	0.89	Yes	6.8	**391**	**117**	**4.7**	**2.6**	8.6	11	172	-	**BAH**	**Empty sella**
E	M 48	8	27.1	98	1.01	No	6.2	**527**	**66.5**	1.6	0.9	15.0	15	183	**-**	**BAH**	Nl
F	M 55	6	38.1	124	0.95	Yes	6.2	**370**	**98**	**7.8**	0.7	36.6	67	75	-	Nl	Nl
G	M 59	10	28.1	102	1.02	Yes	7.3	**346**	**50**	**1.9**	0.3	7.9	122	64	-	Nl	Nl
H	M 62	1	25.5	91	0.92	No	6.3	**386**	**83.4**	1.3	0.9	12.8	178	82	**+**	**BAH**	**6mm nodule**

BAH: Bilateral Adrenal Hyperplasia; Nl: Normal

*** Invalid test;**

Conclusão

A Síndrome de Cushing (SC), que ainda é considerada uma condição clínica rara, sobrepõe-se à síndrome metabólica (SM) em diversos aspectos clínicos, como a hipertensão arterial sistêmica (HAS), a obesidade e a intolerância aos carboidratos ou diabetes mellitus (DM) [10]. A SM cursa com alterações do eixo hipotálamo-hipófise-adrenal [44] e, por sua vez, os pacientes com incidentalomas adrenais apresentam alta freqüência de características da SM [34,35].

Pesquisas prévias demonstraram a presença de SC subclínica entre pacientes com diagnóstico de DM do tipo 2 (DM2) [42,43] e melhora do controle metabólico após o tratamento dessa condição.

Apresentamos neste estudo os resultados da investigação de SC entre 103 pacientes adultos obesos com DM2. Em estudo prévios, os algoritmos sugeridos nas investigações de SC e dos incidentalomas adrenais variam entre os diferentes centros [9,10,27,34].

Essa triagem, em particular, foi realizada com dois diferentes testes e optamos por utilizar como ponto de corte (cut-off) o quintil superior (P80). Desse modo, os resultados acima do P80 (os 20% mais extremos), foram considerados suspeitos para SC.

Exceto pelo cortisol salivar após dexametasona, os valores de corte estabelecidos na presente série para cada teste foram semelhantes aos relatados na literatura. Alterações do eixo HHA relacionadas à obesidade [44], HAS [45] e DM2 [45,46] poderiam justificar resultados falso-positivos, uma vez que

estas condições estavam presentes em 70% dos nossos pacientes. Entretanto, isto é controverso, pois outros autores já demonstraram que a obesidade não é causa de resultado falso-positivo no teste de supressão com dexametasona (TSD) 1mg *overnight* [13,47-49]. Neste estudo, também confirmamos resultados anteriores [43] de que o DM mal controlado não apresenta relação direta com alterações laboratoriais do eixo HHA. Também não encontramos possíveis correlações entre níveis de HbA1c ou a presença de hipertensão. Além disso, outros já demonstraram que o cortisol salivar às 23:00h apresenta sensibilidade e especificidade suficientes para discriminar pacientes com SC daqueles com pseudo-Cushing, assim como de controles obesos e não obesos [21].

Com o objetivo de excluir resultado falso-positivo no TSD com 1 mg, dosamos a dexametasona sérica e consideramos válidos os testes com concentração maior ou igual a 156 ng/dL. Resultados similares (220 ng/dL) já foram demonstrados por outros [50]. Embora sejam essencialmente semelhantes, há algumas diferenças entre nossa população e aquela estudada por Catargi [43]: não utilizamos a HbA1c elevada como critério de inclusão, sendo que a relação da HbA1c com o limite superior do método foi menor no presente estudo, assim como também foram menores o IMC e a relação cintura quadril.

Em nosso estudo oito, entre os 103 pacientes avaliados apresentavam pelo menos dois testes positivos para hipercortisolismo, tendo sido submetidos a exames adicionais. Quando comparados aos pacientes com todos os resultados normais, eles não apresentavam particularidades clínicas, exceto por apresentarem pressão arterial mais elevada. A glicemia e a HbA1c eram

semelhantes senão menores do que no outro grupo. A avaliação combinada das dosagens de cortisol urinário de 24 horas, sulfato de DHEA, ACTH, teste de estímulo com DDAVP, RM de hipófise e CT de adrenais, demonstrou que em cinco deles o diagnóstico de SCS era pouco provável, porém nos três restantes a SC subclínica não pode ser descartada.

Em séries de pacientes com incidentalomas adrenais e SC subclínica, a ausência de manifestações clínicas justifica-se pelas taxas normais de produção de cortisol [28]. Nesta série, os níveis de cortisol urinário foram normais. Uma revisão recente [51] sugere que o cortisol salivar das 23:00h, em duas ocasiões diferentes pode ser a melhor abordagem de triagem para a SC. Entre os pacientes estudados, 3% apresentam alta suspeição de SC subclínica, mas em nenhum este diagnóstico foi confirmado por cirurgia ou anátomo-patológico.

Finalmente, embora não confirmatórios, nossos resultados são muito sugestivos de que a prevalência de SC em sua forma subclínica é significativamente maior em populações de risco do que na população geral, como previamente observado por Leibowitz [42] e Catargi [43].

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