Cardiovascular risk and investigation of glucocorticoid hypersecretion in patients with adrenal tumors

PhD Theses

Márta Sereg, MD

Semmelweis University,
Doctoral School of Clinical Medicine

Consultant: Miklós Tóth, Associate Professor, MD, PhD.

Critical examiners: Csaba Horváth, Associate Professor, MD, PhD, D.Sc.
Kristóf Zupán, MD, PhD.
President of Defence Board: Prof. István Kiss, Full Professor, MD, PhD, D.Sc.
Members of Defence Board: László Kóbori, Associate Professor, MD, PhD.
Zoltán Szentirmay, Centrum Director, MD, PhD.

Budapest
2011

Introduction:

Adrenal incidentalomas are mostly (in 70–80% of cases) non-functioning adrenocortical adenomas which do not cause classical clinical signs and symptoms of hormone excess syndromes (Conn's and Cushing's syndrome). However, 5–47% of the non-functioning adrenal adenomas show mild cortisol oversecretion without symptoms of Cushing's syndrome referred to as subclinical autonomous glucocorticoid hypersecretion (SAGH) or subclinical Cushing's syndrome.

The differential diagnosis of adrenal tumors includes a wide variety of other primary adrenal, periadrenal, and secondary tumors, and even pseudoadrenal and infectious masses. Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. Rarely, tumors with the same histological and immunohistochemical features arise in organs having no connection to the tubular gastrointestinal tract designated as extragastrointestinal stromal tumors (EGISTs).

Determination of midnight salivary cortisol, along with low dose dexamethasone test (LDDST) and urinary free cortisol (UFC) measurement, has become a first-line laboratory method in the diagnosis of Cushing's syndrome. However, there are marked differences between different studies in the sensitivity and specificity of salivary cortisol assays. There is a paucity of reports on the diagnostic utility of salivary cortisol measurements in patients with mild or subclinical Cushing's syndrome and in patients with hormonally inactive adrenal tumors. Bone formation, most frequently evaluated by serum osteocalcin measurement has been known to be greatly suppressed in patients with Cushing's syndrome.

Several lines of evidences suggest that in addition to cortisol-producing adrenal adenomas causing overt Cushing's syndrome, non-functioning adrenal adenomas are also associated with an increased prevalence of metabolic risk factors and increased atherosclerotic risk. Autonomous cortisol hypersecretion may cause a variety of metabolic derangements and disorders, such as arterial hypertension, impaired glucose tolerance and type-2 diabetes mellitus, hypercholesterolemia, hypertrigliceridemia and obesity. These metabolic disorders are well known risk factors for atherosclerosis. An increased prevalence of these metabolic disorders has been reported in patients with subclinical Cushing's syndrome.

It was shown that surgical treatment of endogenous hypercortisolism reduces high blood pressure. Several studies reported on the improvement of the insulin sensitivity and glucose metabolism as well as obesity and lipid metabolism after removal of the adrenal incidentaloma.

However, there is a lack of long-term follow-up studies that investigate the issue of whether patients with incidentally discovered adrenal adenomas, either hormonally inactive or only moderately active, could benefit from adrenalectomy in terms of cardiovascular morbidity and mortality.

Aims of study

A. Atherosclerotic risk factors and complications in patients with non-functioning adrenal adenomas treated with or without adrenalectomy: a long-term follow-up study

1. The first objective was to investigate the prevalence of various atherosclerotic risk factors, both in patients with non-functioning adrenal adenomas treated with and without adrenalectomy.
2. Further aim of this work was to study whether adrenal surgical intervention could have an impact on atherosclerotic morbidity and mortality of patients with non-functioning adrenal adenomas.
3. To investigate the atherosclerotic risk factors as well as atherosclerotic morbidity and mortality in patients with subclinical Cushing's syndrome.

B. Diagnostic performance of salivary cortisol and serum osteocalcin measurements in patients with overt and subclinical Cushing's syndrome

1. The first objective of this study was to investigate the diagnostic applicability of midnight salivary cortisol measurement and to compare its diagnostic performance to other tests including morning and midnight serum cortisol, serum cortisol after LDDST and UFC in a large number of consecutive patients evaluated because of signs and symptoms of Cushing's syndrome or because of the presence of incidentally discovered adrenal tumors.
2. Our further aim was to evaluate the diagnostic utility of serum osteocalcin measurements as compared to that of current methods including salivary cortisol measurements.

C. ***Gastrointestinal stromal tumor presenting as a hormonally inactive adrenal mass***

Our aim was to prove that the histological entity of gastrointestinal stromal tumor should be included in the pathological differential diagnosis of adrenal incidentalomas.

Patients and methods

Patients

1. The results of clinical, laboratory, and imaging investigations of patients with non-functioning adrenal adenomas referred for evaluation between 1990 and 2001 were retrospectively analyzed. 113 patients were re-examined using our re-investigation protocol. In addition, all of the relevant medical documents of 12 further patients (four patients treated and eight not treated with adrenalectomy), who could not attend our clinic for the follow-up examination, were obtained either from the patients or their relatives. Of the 125 patients with non-functioning adrenal adenomas having follow-up examinations or presenting follow-up data, 47 patients underwent unilateral adrenalectomy between 1990 and 2001. The remaining 74 patients were treated conservatively, without adrenalectomy.

2. The study population included a total of 151 patients consecutively referred for endocrinological evaluation. All patients were prospectively examined for the presence of Cushing's syndrome. Based on the final diagnosis, each patient was included into one of the following four groups: (1) group A, patients with overt Cushing's syndrome (n = 23), (2) group B, patients with subclinical Cushing's syndrome (n = 18), (3) group C, patients with hormonally inactive adrenal adenomas (hence without subclinical Cushing's syndrome) (n = 40), (4) group D, patients without HPA disturbances (hypercortisolism excluded, lack of known adrenal tumor; n = 70).

3. A 68-year-old woman presenting with a 3-month history of right abdominal pain was clinically diagnosed as having a hormonally inactive right adrenal tumor sizing 15 cm in diameter.

Methods

1. All patients underwent complete clinical, radiological, and hormonal evaluation during their baseline investigation. Serum cortisol concentrations were measured from blood samples collected at 0800 and 2400 h, as well as after LDDST (1 mg). Hormonal investigations also included the measurements of serum DHEA sulfate, aldosterone, 17-hydroxyprogesterone, testosterone, plasma ACTH concentration, and plasma renin activity. The 24-h urine collection was performed for the determination of vanillylmandelic acid excretion. Nuclear magnetic resonance imaging and cholesterol- or MIBG-scintigraphy were performed if indicated. In case of a suspicion for primary aldosteronism, saline suppression test and, in some patients, selective adrenal vein sampling for aldosterone and cortisol measurements were also performed.

We examined the prevalences of hypertension, type-2 diabetes mellitus, hyperlipidemia and obesity both at baseline and at the time of reinvestigation as well as the incidence of newly-onset angina pectoris, acut myocardial infarction, cerebrovascular stroke or the incident events of percutaneous transluminal coronary angioplasty, coronary bypass surgery, intervention for peripheral arterial stenosis/occlusion during the follow-up period in patients with non-functional adrenal adenomas.

Patients on antihyperlipidemic medications and those who had total cholesterol greater than 5.2 mmol/l, low density lipoprotein cholesterol greater than 2.6 mmol/l or triglycerides greater than 1.7 mmol/l were considered to have hyperlipidemia. Patients with a previous definitive diagnosis of diabetes mellitus and those on antidiabetic medications were regarded as diabetic. All of the other patients underwent an oral glucose tolerance test (OGTT, 75 g glucose diluted in 250 ml water). Diabetes mellitus was diagnosed when fasting plasma glucose exceeded 7.0 mmol/l or the glucose response after OGTT at 120 min was 11.1 mmol/l or greater. IGT was diagnosed when blood glucose levels at 120 min after OGTT were between 7.8 and 11.0 mmol/l. Patients regularly taking antihypertensive medications and patients repeatedly having blood pressure greater than 140/90 mmHg were considered to have hypertension.

Subclinical Cushing's syndrome was diagnosed in patients without overt clinical signs and symptoms of Cushing's disease with at least one of the following two criteria: i) midnight serum cortisol concentration greater than 5 μg/dl and ii) plasma cortisol concentration greater than 3.6 μg/dl after LDDST.

For the comparison of the prevalences of hypertension and diabetes mellitus in our patients to that of the general Hungarian population, a large, community based sample obtained in primary care facilities in four Hungarian counties was used.

2. Serum cortisol concentrations were measured from blood samples collected at 08:00 and 24:00 h, as well as after a LDDST (1 mg). For UFC measurement 24-h urine collection was performed. Salivary cortisol was sampled between 23:00 h and 24:00 h and at 08:00 h in the morning. Patients with hypertension and those with incidentally discovered adrenal adenomas were screened for pheochromocytomas (urinary VMA, metanephrine and normetanephrine excretions) and for primary hyperaldosteronism (plasma aldosterone concentration/plasma renin activity ratio). Blood sample was taken at 08:00 h for plasma adrenocorticotropic hormone (ACTH) measurements in all patients with adrenal tumors and in patients whose initial hormonal findings suggested the possibility of Cushing's syndrome. Serum, salivary and urinary cortisol as well as plasma ACTH were measured by electrochemiluminescence immunoassay according to the instructions of the manufacturer. Blood samples for measurements of serum osteocalcin were collected at 08:00 h after an overnight fast. Serum osteocalcin was measured with kits from Roche Diagnostics according to the manufacturer's instructions.

Body mass index (BMI) >30 kg/m2 was considered an index of obesity.

Subclinical Cushing's syndrome was diagnosed in patients without overt clinical signs and symptoms of Cushing's syndrome who had at least two of the following three criteria: (1) midnight serum cortisol concentration >5.0 μg/dl; (2) plasma cortisol concentration >3.6 μg/dl after LDDST; (3) plasma ACTH concentration <7.2 pg/ml in patients with uni- or bilateral adrenal adenomas/hyperplasias.

Because of the lack of clinically significant differences in serum cortisol at 08:00 h, 24:00 h, midnight salivary cortisol, UFC and serum osteocalcin concentrations between group C and group D, laboratory data obtained from these two groups were combined and used as a control group for receiver–operator characteristic (ROC) analysis.

Statistical analysis

In our first study statistical analysis was performed using Statistica package (version 7.0, Statsoft Inc., Tulsa, Oklahoma, USA). A value of $P<0.05$ was considered to be significant. The relative frequencies of each atherosclerotic event and disease recorded at follow-up were

compared between patient groups treated with and without adrenalectomy, using chi-square analysis and Fisher's exact test. The frequencies of metabolic disorders and hormonal parameters were compared with t-test or Mann–Whitney U-test.

In our second study statistical analysis was performed using SPSS software, version 15 (SPSS 15.0, SPSS Inc., Chicago, IL). Normality of data distribution was analyzed by the Shapiro–Wilk's test. Results are expressed as the mean ± S.D. Associations between different laboratory parameters were determined by linear regression analysis. The differences in biochemical variables between patient groups were evaluated with oneway ANOVA and Bonferroni post hoc test. A value of $p < 0.05$ was considered to be significant. The diagnostic accuracy of various tests was evaluated using ROC analysis. Optimal cut-off point for each test was obtained by calculating the Youden index from ROC analysis plotting patients with overt or subclinical Cushing's syndrome vs. patients without overt or subclinical Cushing's syndrome. Significance was set at $p < 0.05$.

Results

1. There were no statistically significant differences in follow-up times, age and sex ratios between patients treated and not treated with adrenalectomy. However, the baseline diameter of adrenal tumors of patients who underwent adrenalectomy was significantly larger when compared with that found at baseline in patients without subsequent adrenalectomy. At the baseline, endocrine investigation failed to show differences between patients subsequently treated and not treated with adrenalectomy. The prevalences of hypertension at baseline were high in both groups (86 and 80% in patients with and without subsequent adrenalectomy, respectively), and this high prevalence persisted at the time of follow-up without significant differences between patients treated with or without adrenalectomy. At the time of follow-up, BMI was slightly increased in both groups without any significant differences between the two groups. When compared with baseline, the prevalence of hyperlipidemia and the number of patients treated with lipid-lowering drugs increased significantly at the time of follow-up. When compared with baseline, the prevalence of type-2 diabetes mellitus increased, whereas the prevalence of impaired glucose tolerance decreased significantly during the follow-up period in both groups of patients, independently of adrenalectomy. Surgical removal of the adrenal adenoma failed to influence the prevalence of type-2 diabetes mellitus.

When compared with the data obtained from a large cohort representing the Hungarian general population, the prevalences of hypertension and diabetes mellitus were significantly higher in patients with non-functioning adrenal adenomas both at baseline and at the time of follow-up, independently of adrenal surgical intervention. Similarly, no statistically significant differences were found in the prevalences of coronary and peripheral arterial events and interventions between patients treated and not treated with adrenalectomy. In addition, the cardiovascular mortality was also similar in the groups of patients treated and not treated with adrenalectomy.

Thirteen of the 125 patients (10.4%) with non-functioning adrenal adenomas qualified for subclinical Cushing's syndrome. The occurence of metabolic and cardiovascular risk factors was similar in patients with and without subclinical Cushing's syndrome. There were no significant differences in any other laboratory parameters or in the prevalences of incident vascular events and interventions between patients with and without subclinical Cushing's syndrome.

2. The highest cortisol concentrations in serum, saliva and urine were detected in patients with overt Cushing's syndrome (group A) with the exception of salivary cortisol at 08:00 h, which did not differ between the four groups (A-B-C-D). There were no statistically significant differences in serum and salivary cortisol concentrations at 08:00 h and 24:00 h and in 24 h urinary cortisol excretion between patients with hormonally inactive adrenal adenomas (group C) and those without HPA disturbances (group D). Serum osteocalcin concentrations were also similar in groups C and D. However, patients with overt Cushing's syndrome (group A) had significantly suppressed serum osteocalcin concentration as compared to those with subclinical Cushing's syndrome (group B) and the control group. ROC curves were generated and cut-off values were determined for each laboratory parameter to characterize and compare their diagnostic performance for distinguishing patients with overt and subclinical Cushing's syndrome from the control group (group C and D). For the diagnosis of overt Cushing's syndrome, the 95% confidence interval of AUC were similar for salivary cortisol at 24:00 h, serum cortisol at 24:00 h, serum cortisol after LDDST and UFC. The 95% confidence interval of serum osteocalcin was also similar to that of salivary cortisol at 24:00 h and serum cortisol after LDDST for the diagnosis of overt Cushing's syndrome. The diagnostic performance of all these hormonal parameters proved to be inferior for the diagnosis of subclinical Cushing's syndrome compared to that found for the diagnosis of overt Cushing's syndrome.

3. Hormonal evaluation of the patient finally diagnosed with adrenal GIST tumor, failed to show any significant abnormality, therefore, the tumor was considered as a hormonally inactive adrenal tumor. Serum tumor markers were within reference ranges. The 15-cm retroperitoneal tumor fixed to the right adrenal but without having any connection to the gastrointestinal tract was removed during laparotomy. The tumor showed no signs of invasiveness or seedling, and it seemed to be completely excised. Light microscopy confirmed the tight direct connection of the tumor with the histologically normal adrenal gland. Immunohistochemical analysis revealed staining for CD117 and SMA, while beta-catenin, CD34, desmin, S-100 protein, and H-caldesmon staining were absent. The KIT mutation status of the patient was analyzed by direct sequencing of the c-KIT gene. Sequence analysis showed no mutations in KIT gene exons 9, 11, 13, and 17, and in exon 18 of the platelet-derived growth factor-2 receptor gene (PDGFR). Abdominal and chest CT scan performed 3, 9, and 12 months after surgical intervention did not show any sign of residual mass or metastases. At the 18-month follow-up, the patient was without any complaint, and abdominal ultrasonography was also negative. Since pre- and post-operative imaging did not show any sign of residual mass or metastatic disease until the 18th postoperative month, medical oncotherapy was not started.

Discussion

1. The long-term benefit attained from surgical intervention is one of the most important unresolved issues regarding the management of patients with incidentally discovered adrenal tumors. Although the prevalence of metabolic abnormalities and accompanying disorders resulting in an increased atherosclerotic risk are high among patients with non-functioning adrenal adenomas and especially in those causing subclinical hypercortisolism. Our study, in accordance with other reports demonstrates that the prevalences of several atherosclerotic risk factors are higher in patients with non-functioning adrenal adenomas than in the general population. Our results indicate that despite the highly increased atherosclerotic risk factors, surgical removal of the non-functioning adrenal adenoma fails to lower the prevalence of various adverse vascular events. Our results clearly indicate that adrenalectomy performed in patients with non-functioning adrenocortical adenomas failed to normalize or improve the adverse metabolic profile and the increased prevalence of hypertension, type-2 diabetes mellitus, dyslipidemia, and obesity during a 9-year period of postoperative followup.

Adrenalectomy in these patients did not result in a decrease of atherosclerotic morbidity and mortality.

2. Our results with salivary cortisol measurements clearly show that midnight salivary cortisol measured by automated electrochemiluminescence immunoassay had a good diagnostic performance in the clinical setting of the everyday endocrinological praxis and we made a proposal for cut-off points of the hormonal parameters. 5 out of the 7 parameters, namely serum and salivary cortisol at 24:00 h, serum cortisol after LDDST, UFC and serum osteocalcin had diagnostic specificity and sensitivity greater than 90% for the discrimination of patients with overt Cushing's syndrome from patients of the control group. However, only serum and salivary cortisol at 24:00 h and serum cortisol after LDDST had an acceptable but moderate diagnostic performance for the discrimination of patients with subclinical Cushing's syndrome from patients of the control group.

Furthermore, our study shows that serum osteocalcin has a remarkably good diagnostic performance when compared to several other tests used for the diagnosis of overt Cushing's syndrome. Serum osteocalcin significantly correlated with serum and salivary cortisol at 24:00 h, serum cortisol at 08:00 h and with serum cortisol after LDDST in patients with Cushing's syndrome. However, serum osteocalcin of patients with subclinical Cushing's syndrome did not differ from that of control subjects.

3. Mesenchymal tumors sharing immunohistochemical features of GISTs but having no connection to the tubular gastrointestinal tract are designated as extragastrointestinal stromal tumors (EGISTs). Immunohistochemical demonstration of CD117 and/or CD34 is a hallmark of the pathological diagnosis of GIST. The case under study demonstrates that EGISTs should be included in the differential diagnosis of hormonally inactive adrenal masses and that CD 117 [c-KIT] immunohistochemistry is essential for precise and specific diagnosis of GIST among adrenal tumors. Despite the usually large size of these tumors, the prognosis of EGISTs is frequently excellent.

Conclusion

1. Several atherosclerotic risk factors are higher in patients with non-functioning adrenal adenomas than in the general Hungarian population. Adrenalectomy performed in these patients failed to normalize or improve the adverse metabolic profile and the increased prevalence of hypertension, type-2 diabetes mellitus, dyslipidemia and obesity during a 9-year period of postoperative follow-up. Adrenalectomy performed in these patients did not result in a decrease of atherosclerotic morbidity and mortality.

2. Our study confirms the diagnostic applicability of automated electrochemiluminescence immunoassay for midnight salivary cortisol measurements in the diagnosis of overt Cushing's syndrome, even in the clinical setting of the everyday endocrinological praxis. Our results, showing strong correlations between serum cortisol and osteocalcin concentrations, suggest that serum osteocalcin may be considered as a sensitive biological marker of endogenous hypercortisolism, and it may have a role in the diagnosis of overt Cushing's syndrome.

The degree of hypercortisolism in patients with subclinical Cushing's syndrome was not high enough to suppress bone formation and, therefore, serum osteocalcin measurement may not be useful for the diagnosis of subclinical Cushing's syndrome.

3. EGISTs should be included in the differential diagnosis of hormonally inactive adrenal masses.

List of publications

Publications associated with dissertation

1. Sereg M, Szappanos Á, Tőke J, Karlinger K, Feldman K, Kaszper É, Varga I, láz E, Rácz K, Tóth M Atherosclerotic risk factors and complications in patients with non-functional adrenal adenomas treated with or without adrenalectomy: a long-term follow-up study
European Journal of Endocrinology (2009) 160 647-655 **Impact factor:** 3,539

2. Sereg M, Tőke J, Patócs A, Varga I, Igaz P, Szücs N, Horányi J, Pusztai P, Czirják S, Gláz E, Rácz K, Tóth M. Diagnostic performance of salivary cortisol and serum osteocalcin measurements in patients with overt and subclinical Cushing's syndrome
Steriods (2011) 38-42 **Impact factor:** 2,905

3. Sereg M, Buzogány I, Gonda G, Sápi Z, Csöregh É, Jakab Zs, Rácz K, Tóth M. Gastrointestinal stromal tumor presenting as a hormonally inactive adrenal mass
Endocrine (2011) 39 1-5 **Impact factor:** 1,278

Other publications

1. Bekő G, Varga I, Glaz E, Sereg M, Feldman K, Tóth M, Rácz K, Patócs A.
Cutoff values of midnight salivary cortisol for the diagnosis of overt hypercortisolism are highly influenced by methods.
Clinica Chemica Acta 411:(5-6) pp. 364-367. (2010) **Impact factor:** 2,535

2. Szappanos A, Patócs A, Tõke J, Boyle B, Sereg M, Majnik J, Borgulya G, Varga I, Likó I, Rácz K, Tóth M.
BclI polymorphism of the glucocorticoid receptor gene is associated with decreased bone mineral density in patients with endogenous hypercortisolism.
Clinical Endocrinology 71:(5) pp. 636-643. (2009) **Impact factor:** 3,201

3. Majnik J, Patócs A, Balogh K, Luczay A, Török D, Szabó V, Borgulya G, Gergics P, Szappanos A, Bertalan R, Belema B, Tőke J, Sereg M, Nagy ZZ, Sólyom J, Tóth M, Gláz E, Rácz K, Németh J, Fekete G, Tulassay Zs

Nucleotide sequence variants of the glucocorticoid receptor gene and their significance in determining glucocorticoid sensitivity.
Orvosi Hetilap 147:(44) pp. 2107-2115. (2006)