CLINICAL STUDY

Glomerular filtration rate in patients with Cushing's disease: a matched case–control study

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Abstract

Background: Patients with Cushing's disease have a high prevalence of atherosclerosis and maintain an increased cardiovascular risk even after cure of the disease. However, the impact of Cushing's disease on renal function remains to be quantified.

Objectives: To evaluate glomerular filtration rate (GFR) and to identify predictors of GFR in patients with Cushing's disease.

Design and methods: We conducted a matched case–control study: 18 patients with active or cured Cushing's disease were compared with healthy population controls matched for age and sex. The main outcome measures were GFR and micro-albuminuria.

Results: Patients with Cushing's disease had a lower GFR, as measured by 24-h creatinine clearance (79 versus 95 ml/min per 1.73 m², $P = 0.005$) and estimated by the MDRD₂ formula (75 versus 88 ml/min per 1.73 m², $P = 0.008$). Multiple regression analyses indicated that disease duration was the strongest predictor for a worse GFR. The prevalence of micro-albuminuria was low (5.5% in both groups).

Conclusions: Patients with Cushing's disease have a decreased GFR. Even if they are cured, close follow-up with strict control of cardiovascular risk factors and monitoring of GFR seems mandatory. Furthermore, the dosage of certain drugs should be adapted to the individual GFR.

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Introduction

Cushing's disease, a rather rare disease with an incidence of about 2.4 per million people per year, affects women 5 to 8 times more frequently than men, and its peak incidence occurs between ages 25 and 40 years (1–3).

Patients with Cushing's disease have an increased risk for developing cardiovascular disease. Chronic hypercortisolemia is associated with cardiovascular risk factors such as systemic arterial hypertension, impaired glucose tolerance or diabetes mellitus, central obesity with insulin resistance, hyperlipidemia and hypercoagulability. The increased cardiovascular risk can persist a long time after remission of the disease. In this regard, Colao *et al.* (4) reported that patients with a history of Cushing's disease have a high prevalence of atherosclerosis and keep an increased cardiovascular risk even after normalization of circulating cortisol levels for 5 years or more. The same authors provide evidence for the fact that abdominal obesity and/or insulin resistance are the most important factors contributing to this cardiovascular risk (4).

Cardiovascular risk factors also have a major impact on renal function (5). More specifically, the metabolic

syndrome, of which many features are present in Cushing's disease, is a strong and independent risk factor for chronic kidney disease and micro-albuminuria (6).

It is somewhat surprising therefore, that data on renal function in patients with Cushing's disease are scarce (7–10). So far, no studies have examined the potential effect of cardiovascular risk factors on renal function in these patients. If renal function is impaired, and if the factors contributing to renal impairment are identified, this could have therapeutic implications in terms of prevention of renal dysfunction.

We therefore performed a matched case–control study with a 2-fold aim: first, to investigate if patients with Cushing's disease have a decreased glomerular filtration rate (GFR) in comparison to the normal population; secondly, to explore the potential impact of different factors known to be present in glucocorticoid excess on GFR.

Patients and methods

Overall study design and patient recruitment

The design was a matched case–control study. Patients previously diagnosed with Cushing's disease (cases) were matched, with respect to age (± 3 years)

and sex, with healthy subjects from the general population (controls). All subjects gave written informed consent. Criteria for exclusion from the study were a familial or personal history of kidney disease before the onset of the Cushing's disease. The protocol and consent form were approved by the Ethical Committee of the Vrije Universiteit Brussel (Brussels, Belgium).

Patients with Cushing's disease were recruited from three hospitals (Academisch Ziekenhuis van de Vrije Universiteit Brussel, Universitair Ziekenhuis Antwerpen, and Middelheim Ziekenhuis Antwerpen) between March and December 2003. The diagnosis of Cushing's disease, made between 1982 and 2003, was based on: (i) increased diurnal urinary cortisol excretion; (ii) increased basal serum cortisol levels with loss of physiological circadian rhythm with normal or increased plasma adrenocorticotrophic hormone (ACTH) concentrations; (iii) lack of cortisol suppression after administration of low-dose dexamethasone. In all patients, the presence of a pituitary tumor was confirmed by computed tomography or magnetic resonance imaging (MRI) and/or inferior petrosal sinus sampling.

The criteria considered for cure of Cushing's disease were defined by: (i) urinary diurnal cortisol excretion below or within the normal range; (ii) serum cortisol concentrations below or within the normal range; (iii) suppression of serum cortisol concentrations after 1 mg dexamethasone (11).

The following data were extracted from patient files: age at diagnosis, gender, duration of active disease, clinical features at diagnosis (including appearance of weight increase, central fat distribution, moonface, buffalo hump, purple striae, ecchymosis, thinning of the skin, myopathy, edema, hirsutism, acne, menstrual disorders, headache, blurred vision, psychiatric disorders, hypertension, diabetes mellitus and/or impaired glucose tolerance, pituitary deficiency, osteoporosis, and presence of nephrolithiasis), 24-h cortisoluria and plasma ACTH and cortisol levels at diagnosis, results of imaging studies, outcome of surgery, additional radiation therapy, surgery and/or specific medication.

We also documented the current status of Cushing's disease (cured or active), smoking status and the current use of medication.

Clinical and biochemical measurements

Current height, weight, body mass index (BMI), waist and measurements of heart rate, systolic blood pressure, and diastolic blood pressure were evaluated by standard methods in both groups, i.e. among cases (patients who have suffered or have Cushing's disease) and controls (healthy persons from the general population). Overweight was defined as a BMI of

25–29.9 kg/m² and obesity as a BMI $\geq$ 30 kg/m² (12). Blood pressure was measured in a relaxed sitting position with a mercury sphygmomanometer. Hypertension was diagnosed when systolic blood pressure was more than 140 mmHg and/or diastolic blood pressure was more than 90 mmHg (13).

Creatinine clearance was computed from creatinine excretion in a 24-h urine collection and a single measurement of serum creatinine. Plasma and urine creatinine were measured in the same laboratory by using the Vitros enzymatic creatinine assay (Ortho-Clinical Diagnostics, Inc., 100 Indigo Creek Drive, Rochester, NY, USA) with a normal range in plasma of 0.5–1.5 mg/dl in adult men and 0.4–1.2 mg/dl in women. The precision of the assay was characterized by a coefficient of variation of 1.7% at 0.87 mg/dl and 1.3% at 5.95 mg/dl during the study. The accuracy of the assay was characterized by a coefficient of variation of 8.7% at 0.85 mg/dl, 0.9% at 0.90 mg/dl, 2.9% at 0.99 mg/dl, 3.4% at 1.96 mg/dl, 1.5% at 2.00 mg/dl and 3.7% at 6.30 mg/dl. The z-scores for accuracy, ranging between –0.69 and 1.19, provided no evidence for a statistically significant over- or underestimation of plasma creatinine measurements compared with the external reference standard of the Federal Government (Federale Overheidsdienst Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu, Externe Kwaliteitsevaluatie, Juliette Wytmanstraat 14, Brussels; <http://www.iph.fgov.be>). The assay had very stable quality control measures during the duration of the study (between-day imprecision of less than 2.5% at levels of 0.65 and 7.59 mg/dl, Laboratory Procedures Manual 2003, Academisch Ziekenhuis van de Vrije Universiteit Brussel).

The serum and urine specimens were also used for other measurements. Plasma renin, aldosterone, insulin, ACTH and serum and urinary cortisol were assayed by immunoradiometric assay (IRMA) or by radioimmunoassay. Plasma urea, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, hemoglobin, hematocrit, red blood cells, glucose, hemoglobin A1c and electrolytes were measured by standard laboratory procedures. Calciuria and urinary creatinine were quantified on a 24-h urine collection. Completeness of urinary collection was determined by creatinine excretion over 24 h.

Urinary micro-albumin was measured on a 24-h urine collection, using an immuno-turbidimetric method (Roche-Diagnostics), and was considered positive if it exceeded 30 mg/24 h

Assessment of GFR (primary outcome measure)

In both groups GFR was evaluated by several non-invasive methods.

Twenty-four-hour creatinine clearance was calculated using the following formula:

$$\begin{aligned} \text{Creatinine clearance} &= \text{urinary creatinine (mg/dl)} \\ &\quad \times 24\text{-h urinary volume (ml/min)} / \\ &\quad \text{serum creatinine (mg/dl)} \end{aligned}$$

We also normalized the 24-h creatinine clearance for a body surface of 1.73 m², using the Mosteller formula for body surface area (14):

$$\begin{aligned} \text{Body surface area (m}^2\text{)} &= [\text{height (cm)} \times \text{weight (kg)} / \\ &\quad 3600]^{1/2} \end{aligned}$$

The Cockcroft–Gault formula was also used to estimate GFR:

$$\begin{aligned} \text{GFR (ml/min)} &= [140 - \text{age (years)}] \times \text{weight (kg)} \\ &\quad \times (0.85 \text{ if woman}) / \\ &\quad [72 \times \text{plasma creatinine (mg/dl)}] \end{aligned}$$

Cockcroft–Gault estimates of GFR were also normalized for a body surface of 1.73 m².

GFR was also estimated by using the simplified Modification of Diet in Renal Disease Study equation (MDRD₂), defined as follows (15, 16):

$$\begin{aligned} \text{GFR (ml/min per 1.73 m}^2\text{)} \\ &= 186 \times \text{serum creatinine}^{-1.154} \\ &\quad \times \text{age}^{-0.203} \times (0.742 \text{ if woman}) \\ &\quad \times (1.210 \text{ if Afro-American}) \end{aligned}$$

Statistical analyses

Data on demographic characteristics, clinical and biochemical measurements were summarized descriptively for both groups, i.e. patients with Cushing's disease and healthy controls. Differences in the mean values of continuous variables among the matched groups were evaluated by the paired Student's *t*-test for normally distributed variables and Wilcoxon signed ranks test for other variables. No adjustments for multiple comparisons were made to the reported *P* values. Readers become able therefore, if they so desire, to do a Bonferroni (or other) adjustment for the multiplicity.

Next, a one-way between-groups multivariate analysis of variance (MANOVA) was performed to investigate differences in GFR between the group of patients with Cushing's disease and the control group.

Five dependent variables (primary outcome measures) were used in the MANOVA model: 24-h

creatinine clearance, 24-h creatinine clearance normalized for a body surface of 1.73 m², GFR estimated by the Cockcroft–Gault formula, GFR estimated by the Cockcroft–Gault formula normalized for a body surface of 1.73 m² and GFR calculated according to the MDRD₂ formula. Given the small sample size of our pilot study we performed statistical tests on log-transformed data for the five dependent variables. To facilitate clinical interpretation, the results were re-expressed in terms of the original units.

The independent variable of the MANOVA model was defined by group, i.e. patients with Cushing's disease versus healthy controls.

The significance of the overall MANOVA model was assessed by Wilk's lambda statistic, the *F* statistic and the corresponding *P* value. Only a single test is performed to assess whether the five dependent variables (as a cluster) differ among patients with Cushing's disease versus healthy controls. To assess whether each individual outcome measure was statistically different among groups, we chose to regard a level of *P* < 0.01 as significant for these individual comparisons, representing a Bonferroni correction of 0.05 divided by the five outcome measures of primary interest.

Finally, we used multiple regression analyses to investigate the joint effect and independent contribution of several factors (explanatory variables) on GFR (dependent variables) among patients with Cushing's disease.

The five primary outcome measures – i.e. GFR measured by a 24-h creatinine clearance and normalized for a body surface of 1.73 m², GFR estimated by the Cockcroft–Gault formula and normalized for a body surface of 1.73 m², and GFR calculated using the MDRD₂ formula – were modeled separately.

A series of multiple regression analyses were conducted to identify and confirm the combination of variables that was most effective for predicting each outcome measure of primary interest. The following variables were considered for inclusion in the multiple regression models as possible predictors of each outcome variable of interest: gender, duration of active disease, weight increase, smoking status, pituitary deficiency and age at diagnosis; current BMI, waist, heart rate, systolic blood pressure, diastolic blood pressure, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides and glucose levels; current antihypertensive and hyperlipemic drug use. These variables describe patient characteristics at diagnosis considered to be useful to predict or confound renal function, and well-known risk factors for cardiovascular disease. To determine which of these candidate variables might be useful in the multiple regression models, the association between each candidate variable and each outcome measure of interest was assessed separately. Stepwise multiple regression calculations were then performed with candidate variables entered that showed *P* values < 0.05 when assessed separately.

For each multiple regression model the significance of the overall model was assessed by the *F* statistic. This was because only a single test is performed to assess whether the independent variables (as a group) are associated with the outcome without worrying about adjusting for multiple comparisons. To assess whether the individual independent variables from each multiple regression model were statistically associated with the outcome of interest, the readers become able, if they so desire, to do a Bonferroni (or other) adjustment for the multiplicity. To calculate the Bonferroni correction the usual *P* value (e.g. 0.05) is simply divided by the number of independent (predictor) variables in the model.

All data analyses were performed using SPSS version 12.0 (SPSS, Inc., Chicago, IL, USA).

Results

Characteristics of patients with Cushing's disease

We enrolled 18 patients previously diagnosed with Cushing's disease: 3 patients with active Cushing's disease (2 men, 1 woman; mean age 51 years) and 15 patients cured of Cushing's disease (3 men, 12 women; mean age 45 years).

The initial diagnosis of Cushing's disease was based on three diagnostic tests in eight patients (a low-dose dexamethasone test, MRI and sinus petrosus sampling), and on two tests in ten patients (low-dose dexamethasone test and MRI). The clinical features of the patients at initial diagnosis are shown in Table 1.

All 18 patients had undergone transsphenoidal pituitary adenectomy one or more times. In 12 patients this was unsuccessful, and additional therapy was needed. Additional therapy included pituitary irradiation

(stereotactic and classical) and chemical or surgical adrenalectomy.

Upon entry in the current case-control study, 15 out of 18 patients with Cushing's disease were declared cured; of these 15 patients, the 8 patients who had developed hypocorticism were treated by corticosteroid substitution (hydrocortisone, 20–30 mg daily). Three patients suffered from active Cushing's disease: 1 patient had hypercortisolemia, and 2 patients showed normal cortisol levels under treatment. At study entry, secondary hypopituitarism was observed in 13 patients. All patients with secondary pituitary deficiencies were treated with hormonal replacement at the appropriate doses. Other clinical features of the patients with Cushing's disease upon entry in the current case-control study were: arterial hypertension in 5 patients (1 patient was treated by an angiotensin-converting enzyme (ACE)-inhibitor, the other patients received other antihypertensive drugs), diabetes mellitus in 1 patient, and medication for dyslipidemia in 5 patients. Three patients were smokers. Seven patients were overweight and 2 patients were obese (compared with 5 overweight people in the control group).

Clinical and biochemical data: patients with Cushing's disease versus controls

Table 2 shows the current characteristics of the patients with Cushing's disease (cases) and the matched healthy individuals (controls) upon entry in our case-control study. In the group with Cushing's disease, height was significantly lower ($P = 0.003$) than in the controls. Weight, BMI, waist, blood pressure and heart rate at rest were not significantly different between the two groups.

Patients with Cushing's disease had significantly higher plasma creatinine ($P = 0.017$) and plasma urea ($P = 0.014$) levels, and significantly lower 24-h calciuria ($P = 0.002$), hematocrit ($P = 0.022$), total red blood cells ($P = 0.017$) and glucose ($P = 0.026$) levels than the controls. Blood lipid levels, hormonal levels (renin, aldosterone, insulin, ACTH, serum cortisol and cortisoluria), hemoglobin A1c and serum electrolytes were not significantly different between the two groups.

Micro-albuminuria was present in one patient with Cushing's disease, and in one patient from the control group (5.5% in both groups).

When the group of patients with Cushing's disease was stratified according to corticosteroid substitution therapy, the eight patients taking hydrocortisone were older (53 versus 42 years, $P = 0.038$), had lower plasma cortisol (66 versus 227 $\mu\text{g/l}$, $P = 0.002$) and higher plasma urea levels (50 versus 31 mg/l , $P = 0.013$).

Table 1 Clinical characteristics of patients with Cushing's disease at diagnosis.

Clinical feature	Number (%)
Weight increase	16/18 (89%)
Central fat distribution	11/18 (61%)
Moonface/buffalo hump	12/18 (67%)
Thin skin with easy bruising	6/18 (33%)
Striae	6/18 (33%)
Myopathy	6/18 (33%)
Edema	6/18 (33%)
Hirsutism*	10/13 (77%)
Menstrual disorders*	8/13 (61%)
Headache/blurred vision	9/18 (50%)
Psychiatric disorders	5/18 (28%)
Hypertension	9/18 (50%)
Diabetes mellitus/glucose intolerance	2/18 (11%)
Osteoporosis	3/18 (17%)
Nephrolithiasis	3/18 (17%)

* Applicable to women only.

Table 2 Current clinical and biochemical characteristics of 18 patients with Cushing's disease and 18 healthy population controls.*

	Cushing's disease (cases)	Healthy individuals (controls)	P value
Age (years)	47 (12)	47 (12)	1.000
Height (cm)	163 (7)	169 (8)	0.003
Weight (kg)	73 (19)	72 (14)	0.903
Body surface (m ²)	1.80 (0.22)	1.83 (0.20)	0.642
BMI (kg/m ²)	27.7 (8.1)	25.2 (3.5)	0.273
Waist (cm)	94 (18)	89 (12)	0.372
Heart rate (pulses/min)	76 (13)	77 (7)	0.767
Systolic blood pressure (mmHg)	124 (15)	123 (14)	0.691
Diastolic blood pressure (mmHg)	79 (8)	76 (8)	0.299
Renin (ng/l)	9.2 (5.9)	10.5 (5.8)	0.971
Aldosterone (ng/l)	232 (460)	116 (58)	0.368
Insulin (mIU/l)	6.65 (2.78)	7.21 (5.18)	0.854
ACTH (ng/l)	94 (185)	29 (20)	0.162
Serum cortisol (μg/l)	156 (118)	169 (77)	0.606
24-h urinary volume (ml/day)	1863 (666)	2131 (995)	0.478
24-h urinary creatinine (g/day)	1.14 (0.21)	1.18 (0.25)	0.692
24-h urinary calcium (mg/24 h)	118 (48)	192 (88)	0.002
24-h urinary cortisol (μg/24 h)	66 (42)	48 (17)	0.086
Plasma creatinine (mg/dl)	0.98 (0.24)	0.84 (0.13)	0.017
Plasma urea (mg/dl)	39 (17)	27 (5)	0.014
Total cholesterolemia (mg/dl)	215 (38)	215 (34)	0.930
HDL-cholesterolemia (mg/dl)	65 (17)	62 (17)	0.674
LDL-cholesterolemia (mg/dl)	127 (33)	130 (29)	0.868
Triglycerides (mg/dl)	102 (44)	116 (49)	0.373
Hemoglobin (g/dl)	13.8 (1.1)	14.3 (0.9)	0.143
Hematocrit (%)	41.1 (3.3)	43.6 (2.6)	0.022
Red blood cells (10 ⁶ /mm ³)	4.5 (0.4)	4.9 (0.4)	0.017
Glycemia (mg/dl)	81 (14)	92 (11)	0.026
HbA1c (%)	5.3 (0.4)	5.2 (0.4)	0.941
Sodium (mEq/l)	142 (2)	142 (0.2)	0.755
Potassium (mEq/l)	4.2 (0.3)	4.1 (0.2)	0.442
Chloride (mEq/l)	104 (3)	104 (2)	0.522
Bicarbonate (mEq/l)	28 (2)	29 (1)	0.140

* All values are means (S.D.) unless otherwise stated. Differences among the matched groups were evaluated by the paired Student's *t*-test for normally distributed variables and Wilcoxon signed ranks test for other variables.

GFR: patients with Cushing's disease versus controls

Primary endpoints for GFR are summarized in Table 3. A one-way, between-groups MANOVA showed that there was a statistically significant difference between patients with Cushing's disease (cases) and healthy controls for the combined dependent variables (Wilks' lambda = 0.607; $F_{5,30} = 3.89$, $P = 0.008$). The value obtained for partial eta squared is 0.393. Partial eta squared indicates the magnitude of the differences between means. Partial eta squared, also known as the MANOVA effect size statistic or the 'strength of association', represents the proportion of the variance in the dependent variable (i.e. the five outcome measures of GFR, considered as a cluster) that can be explained by the independent variable (Cushing's disease). To interpret the strength of eta squared values the following guidelines from Cohen (17) can be used: 0.01 = small effect; 0.06 = moderate effect; 0.14 = large

effect (17). The value of 0.393, is thus considered quite a large effect. This means that in our pilot study 39.3% of the variance in GFR can be explained by the presence of Cushing's disease. When the results for the dependent variables were considered separately, and using a Bonferroni adjusted alpha level of 0.010 (= 0.05 divided by 5), the following differences reached statistical significance: GFR calculated from 24-h creatinine clearance, 24-h creatinine clearance normalized for a body surface of 1.73 m² and GFR estimated by the MDRD₂ formula. No statistical difference could be demonstrated for the GFR as estimated by the Cockcroft–Gault formula.

As most patients had been cured of Cushing's disease, we were interested to know whether analyzing only this subgroup would still give the same results. In this regard, a MANOVA analysis performed in the subgroup of 15 patients cured of Cushing's disease upon entry in the current study yielded results essentially identical to those for the full group (data not shown).

Table 3 GFR among 18 patients with Cushing's disease and 18 healthy controls.*

Method used to estimate glomerular filtration rate (dependent variable)	Cushing's disease (cases)	Healthy individuals (controls)	P value
24-h creatinine clearance (ml/min)	82 (20)	99 (17)	0.005
24-h creatinine clearance/1.73 m ² (ml/min per 1.73 m ²)	79 (18)	95 (16)	0.006
Cockcroft–Gault (ml/min)	88 (33)	101 (27)	0.108
Cockcroft–Gault per 1.73 m ² (ml/min per 1.73 m ²)	85 (23)	95 (21)	0.155
MDRD ₂ (ml/min per 1.73 m ²)	75 (16)	88 (15)	0.008

Significance of the overall MANOVA model: Wilks' lambda = 0.607, $F_{5,30} = 3.89$, $P = 0.008$. Importance of the impact of Cushing's disease on GFR: partial eta squared value for the overall MANOVA model = 0.393.

When the results for each dependent variable estimating GFR are then considered separately, a Bonferroni adjusted alpha level of 0.010 (0.05 divided by 5) might be used, and readers are advised to consider only smaller P values as potentially important.

* All values are means (s.d.), unless otherwise stated.

Glomerular filtration was also evaluated in the following subgroups of patients with Cushing's disease: diabetes versus no diabetes and hypertension versus no hypertension at the time of diagnosis; and, upon entry in the current study, cured versus active disease and hydrocortisone treatment versus no corticosteroid substitution. We could not demonstrate any statistical difference between these subgroups with regard to GFR (data not shown).

Although our study was not designed to investigate changes of GFR over time, we were able to take advantage of the data from six patients with Cushing's disease whose GFR was calculated at the time of diagnosis. During a mean ($\pm$ s.d.) follow-up period of 11.3 ± 6.9 years the mean ($\pm$ s.d.) 24-h creatinine clearance dropped from 107 ± 20 ml/min at initial diagnosis of Cushing's disease to 84 ± 22 ml/min upon entry in the current case–control study. With the numbers available, this slight decline in 24-h creatinine clearance of -2.3 ml/min per year among patients diagnosed with Cushing's disease is not statistically significant (paired t -test: $P = 0.08$, degrees of freedom = 5).

Among the patients with Cushing's disease we identified four patients (22%) with chronic kidney disease, i.e. GFR < 60 ml/min per 1.73 m² (18). Table 4 lists the complete description of the characteristics of these four patients, including the risk factors for cardiovascular disease and the GFR estimates. As a result of these GFR estimates, the following investigations were performed: albumin–creatinine ratio in a random untimed urine specimen, examination of the urine sediment for erythrocytes and leukocytes, and imaging of the kidneys by ultrasonography. One patient (VF, Table 4) had bilateral millimetric nephrolithiasis with negative urine sediment and albuminuria. His calcium-phosphorus metabolism was normal. Two patients (VM and WJ, Table 4) had bilateral nephroangiosclerosis as shown on echographic evaluation (small kidneys and decreased cortex size). Both patients were treated for cardiovascular risk factors and ACE inhibition. One patient (BM, Table 4) had normal urine sediment and ultrasonography.

Predictors of GFR among patients with Cushing's disease

Multiple regression analysis showed that the duration of the active disease was the best predictor for glomerular filtration, as measured or calculated by any of the different methods (Table 5). Total cholesterolemia was a significant predictor for 24-h creatinine clearance (Table 5, models 1 and 2). Waist size was a significant predictor for glomerular filtration, as estimated by both the 24-h creatinine clearance and the Cockcroft–Gault formula (Table 5, models 1 and model 3 respectively).

Similar multiple regression analyses performed in the subgroup of 15 patients cured from Cushing's disease upon entry to the current study yielded results essentially identical to those for the full group (data not shown).

Discussion

To our knowledge, our pilot study is the first to report a slight but statistically significant decrease in GFR among patients with Cushing's disease. In addition, we found that disease duration is the strongest predictor for GFR.

The sample of patients we included was representative of the general Cushing's disease population. When comparing clinical features at diagnosis with the data described in the literature, we observed similar clinical signs, although some more specific symptoms – i.e. thin skin with easy bruising and osteoporosis – were less prevalent (19, 20). This discrepancy may be explained by the retrospective nature of our study. Also, both of the other studies were conducted in the early 1980s, when the clinical picture of Cushing's disease was usually more pronounced at the time of diagnosis.

Comparing current laboratory values, patients with Cushing's disease had a lower calciuria, hematocrit and number of red blood cells. These unexpected findings have no clear-cut explanation. The lower blood glucose in cases compared with controls might be

Table 4 Characteristics of the 4 patients with Cushing's disease having chronic kidney disease stage 3 (GFR <60 ml/min per 1.73 m²) (18).

	Patient VM	Patient VF	Patient BM	Patient WJ
Characteristics upon inclusion in the current study				
Gender	Man	Man	Woman	Man
Age at diagnosis of Cushing's disease (years)	53	35	38	62
Age upon inclusion in current study (years)	67	56	45	74
Duration of the active disease (years)	13	20	3	16
Current status of Cushing's disease (cured or active)	Still active	Still active	Cured	Cured
Smoking status	Non-smoker	Non-smoker	Non-smoker	Non-smoker
Diabetes mellitus and/or impaired glucose tolerance	No	No	No	No
BMI (kg/m ²)	29.8	25.6	27.6	20.9
Waist (cm)	115	92	95	74
Systolic blood pressure (mmHg)	135	140	120	120
Diastolic blood pressure (mmHg)	74	80	70	80
Total cholesterolemia (mg/dl)	248	174	253	304
HDL-cholesterolemia (mg/dl)	65	75	67	102
LDL-cholesterolemia (mg/dl)	147	84	137	180
Triglycerides (mg/dl)	180	75	24	106
Hemoglobin (g/dl)	13.8	14.2	15.7	12.1
Hematocrit (%)	41.1	42.2	47.3	36.9
Glycemia (mg/dl)	107	89	82	84
HbA1c (%)	5.8	5.2	5.2	5.3
Plasma creatinine (mg/dl)	1.45	1.35	1.06	1.10
Plasma urea (mg/dl)	63	67	31	44
Presence of microalbuminuria (> 30 mg/24 h)	No	No	No	No
GFR				
24-h creatinine clearance (ml/min)	58.3	62.0	54.0	48.1
24-h creatinine clearance per 1.73 m ² (ml/min per 1.73 m ²)	49.9	57.4	54.3	52.3
Cockcroft–Gault (ml/min)	61	65	73	47
Cockcroft–Gault per 1.73 m ² (ml/min per 1.73 m ²)	52.2	59.9	73.1	51.1
MDRD ₂ (ml/min per 1.73 m ²)	52	58	60	70
Yearly decline in 24-h creatinine clearance (ml/min per year)	NA	–2.52	–6.43	NA

related to hypoadrenalism in this group. Increased levels of insulin were not observed. This is probably related to the fact that BMI and waist were not significantly different compared with the controls. Cholesterol levels were not different when Cushing's disease patients and controls were compared. This is probably a drug effect, as most patients suffering from dyslipidemia were on statin therapy.

We observed no increased prevalence of micro-albuminuria among patients with Cushing's disease. The reason for the low prevalence of micro-albuminuria in the presence of low GFR in patients with Cushing's disease could indicate that the mechanisms responsible for micro-albuminuria are reversible after normalization of cortisol levels. In line with our findings, Koh *et al.* (8) studied 13 patients with Cushing's disease of whom 4 had a pituitary adenoma and 9 an adenoma or carcinoma of the adrenal gland: 80% of the patients with active disease suffered from albuminuria, but this phenomenon was almost completely reversed after normalization of endogenous hypercortisolemia.

Our finding, that patients with a history of Cushing's disease have a significantly lower GFR measured by creatinine clearance and the MDRD₂ formula, is in accordance with the limited data published so far. In

two previous studies Faggiano *et al.* (9, 10) determined the creatinine clearance using a 24-h urine collection. In both studies they reported that patients with Cushing's disease (active and cured) showed a lower clearance than controls, but the difference was not statistically significant. When we compare our study results with those of Faggiano *et al.* (9, 10) we notice that their control group did not include overweight individuals. When GFR was estimated by the Cockcroft–Gault equation we found no statistical differences between our cases and controls. We also found that our Cockcroft–Gault estimates for GFR had quite large standard deviations. A large standard deviation results from data with substantial variability, and so the ability to detect differences between groups becomes less obvious. Poor statistical power is further indicated if sample size is small and if only one outcome of primary interest (GFR estimated according to the Cockcroft–Gault equation) is considered separately for statistical testing. Moreover, the Cockcroft–Gault equation takes into account body weight, another potential source of random measurement error. These findings are in line with the current concept that the MDRD₂ equation is more accurate and precise than the Cockcroft–Gault equation for individuals with a GFR of less than 90 ml/min per 1.73 m² (16, 18).

Table 5 Multiple regression analyses of potential predictors of GFR among patients with Cushing's disease.*

Models with potential predictors for the GFR	Regression coefficient (S.E.)	P value of regression coefficient
Model 1		
Disease duration (years)	-1.55 (0.58)	0.020
Waist (cm)	0.41 (0.18)	0.038
Cholesterolemia (mg/dl)	-0.23 (0.09)	0.020
Predictors for the GFR measured by the 24-h creatinine clearance		
Adjusted <i>R</i> squared = 58%, $F_{3,13} = 8.431$, overall model <i>P</i> value = 0.002		
Model 2		
Disease duration (years)	-1.84 (0.51)	0.003
Waist (cm)	0.08 (0.15)	0.614
Cholesterolemia (mg/dl)	-0.19 (0.08)	0.027
Predictors for the GFR measured by the 24-h creatinine clearance normalized for a body surface of 1.73 m ²		
Adjusted <i>R</i> squared = 59%, $F_{3,13} = 8.619$, overall model <i>P</i> value = 0.002		
Model 3		
Disease duration (years)	-2.64 (0.98)	0.018
Waist (cm)	1.24 (0.30)	0.001
Cholesterolemia (mg/dl)	-0.03 (0.15)	0.829
Predictors for the GFR estimated by the Cockcroft–Gault equation		
Adjusted <i>R</i> squared = 59%, $F_{3,13} = 8.652$, overall model <i>P</i> value = 0.002		
Model 4		
Disease duration (years)	-2.45 (0.76)	0.007
Waist (cm)	0.48 (0.23)	0.058
Cholesterolemia (mg/dl)	-0.06 (0.12)	0.611
Predictors for the GFR estimated by the Cockcroft–Gault equation and normalized for a body surface of 1.73 m ²		
Adjusted <i>R</i> squared = 47%, $F_{3,13} = 5.625$, overall model <i>P</i> value = 0.011		
Model 5		
Disease duration (years)	-1.49 (0.70)	0.052
Waist (cm)	-0.05 (0.21)	0.817
Cholesterolemia (mg/dl)	-0.00 (0.15)	0.977
Predictors for the GFR estimated by the MDRD ₂ equation		
Adjusted <i>R</i> squared = 11%, $F_{3,13} = 1.668$, overall model <i>P</i> value = 0.223		

* For each model, the significance of the overall model is assessed by the *F* statistic and its corresponding *P* value. To assess whether the individual predictor (independent) variables from each multiple regression model are statistically associated with the outcome of interest, the readers become able, if they so desire, to do a Bonferroni (or other) adjustment for the multiplicity. We suggest considering a level of $P < 0.0167$ as significant for these numerous comparisons, representing a Bonferroni correction of 0.05 divided by three predictor variables.

Using multiple regression analyses, the GFR (estimated by the different methods) was consistently influenced by the duration of the active disease. Overall, our analyses point to a deleterious effect of hypercortisolemia on GFR, with a persistently decreased GFR, even after cure of Cushing's disease. From a physiopathological point of view these findings are sound (21–29). Although acute corticosteroid administration in normal men does not modify creatinine clearance, these data do not reflect hemodynamic changes of chronic endogenous hypercortisolism that can influence (deteriorate) GFR (30). Indeed, chronic hypercortisolism influences vascular tone and may permanently alter vessel remodeling.

Arterial hypertension was not identified as a factor predictive of GFR in the current study. Hypercholesterolemia and abdominal adiposity, on the other hand, were identified as independent predictors, but their impact varied according to the method used to evaluate GFR. When plotting the relationship between weight and GFR, it has recently been shown that the

Cockcroft–Gault and MDRD₂ estimates display a different and opposite pattern. For a greater weight, Cockcroft–Gault estimates were higher; whereas MDRD₂ estimates were lower. Since both groups, patients with Cushing's disease and controls, had a similar BMI these findings cannot account for the differences in GFR between our study groups. They can however explain the different impact of waist measurement (correlated to BMI) on estimated creatinine clearance: the higher the waist measurement the higher the Cockcroft–Gault estimates, the higher the waist measurement the lower the MDRD₂ estimates (31). Treatment of cardiovascular risk factors may have confounded these observations further. Most patients were followed closely in a tertiary referral setting by endocrinologists aware of the treatment of these risk factors. Five out of 18 patients were treated for arterial hypertension, and 5 out of 18 received an antilipemic drug. Even then, hypercholesterolemia was a strong and independent predictor of creatinine clearance. In line with these findings, it has recently been demonstrated that the

interpretation of the relationship between cardiovascular risk factors and kidney function is affected by the method used to estimate kidney function. Indirect measures (Cockcroft–Gault, MDRD₂) should therefore be used with caution (31). Finally, visceral adiposity is common in patients with Cushing's disease, mainly because glucocorticoids induce fat accumulation in the visceral area. Colao *et al.* (4) demonstrated that this feature is not completely reversible in patients with Cushing's disease. The decrease of GFR in people with a history of Cushing's disease might be a consequence of the metabolic syndrome that has been shown to affect these people (32, 33).

The strengths of our study include feasibility (related to study design), the inclusion of an adequate control group, the precision and accuracy of the creatinine measurement used in our calculations and the power of the statistical methodology.

A prospective cohort study on kidney failure among patients with Cushing's disease might not be feasible taking into account the low prevalence of Cushing's disease, and the rare occurrence of the main outcome of interest (i.e. progression to kidney failure). Moreover, a prospective outcome comparison between patients with Cushing's disease and control patients would require a very long follow-up period. Another possible study design is the prospective evaluation of patients treated with corticosteroids. In these patients, however, different factors related to the underlying (and treated) disease may confound the outcome measure (i.e. GFR).

We balanced both groups prognostically by taking into account age and gender, which have been shown to be strong and independent predictors for kidney function.

It is well recognized that creatinine measurements from different laboratories using the same samples may differ systematically by as much as 0.23 mg/dl. The *z*-scores for accuracy provided no evidence for a statistically significant over- or underestimation of plasma creatinine measurements compared with the external reference standard of the Federal Government. Consecutive evaluations of precision and accuracy during the study period (March–December 2003) did not show any significant trend.

MANOVA performs only a single test to assess whether several outcome (dependent) variables (as a cluster) are associated with the exposure (independent) variables without worrying about adjusting for multiple comparisons. Likewise, multiple regression analyses investigate the independent effect of several prognostic factors (explanatory variables) on one outcome measure (dependent variable), also without worrying about adjusting for multiple comparisons. Both statistical methods allow for the quantification of the importance of the impact of the independent variable(s) on the dependent variable(s) by using an effect size statistic (partial eta squared and adjusted *R* squared, for

MANOVA and multiple regression analysis respectively).

We did not assess the glomerular function of our participants by the gold standard methodology, i.e. by using the exogenous filtration marker inulin or any other validated filtration marker including, for example, iothexolon or iodine-125-iothalamate. In addition, we did not attempt to assess renal blood flow, renal vascular resistance or filtration fraction. Instead, in our pilot study, we have focused on GFR and used non-invasive methods. In the current study the magnitude of the impact of Cushing's disease on GFR was evaluated by partial eta squared, the effect size statistic of the MANOVA model. The value in our study is 0.393, which, according to generally accepted criteria (17), is considered quite a large effect. This means that in our study 39.3% of the variance in GFR is explained by the presence of Cushing's disease. Advantages for the use of creatinine as a filtration marker are that it is easy to measure, inexpensive and widely available. Irrespective of GFR, however, there are a number of factors affecting serum creatinine levels: increased muscle mass (increased creatinine levels), age, female gender, muscle wasting (muscle mass is lowered in patients with Cushing's disease), a vegetarian diet and malnutrition (decreased creatinine levels). Although inter-group comparison may be valid, the Cockcroft–Gault and MDRD₂ formulae were not designed or validated to estimate near-normal GFRs. The Cockcroft–Gault formula was derived in 249 hospitalized white men, and the MDRD₂ formula in 1628 participants with chronic kidney disease (GFR, 40 ml/min per 1.73 m² or less) who were predominantly white. Both formulae have not been validated in: individuals at the extremes of body size, with high levels of dietary meat intake, overweight or obesity; individuals at increased risk of chronic kidney disease; conditions associated with muscle wasting (e.g. patients with Cushing's disease); normal individuals (e.g. population-based healthy controls). These equations may be less accurate in individuals who were not included in the study population from which the equation was developed or validated. According to current guidelines, however, the most appropriate estimate of GFR in these special circumstances is a measurement of creatinine clearance, obtained from a 24-h urine collection to measure creatinine excretion rate and a single measurement of plasma creatinine concentration (34, 35).

As we did not use a prospective cohort design for the current investigation, the decline of GFR over time could not be studied appropriately. In a prospective cohort study, changes of GFR over time could help to individualize a subgroup of patients particularly at risk for a rapidly progressive decline in GFR.

Our study was also limited by the small number of patients and the fact that most patients were cured upon enrolment in the current case–control study. In

particular, we could not perform a separate evaluation of the different risk factors in the active stage of the disease. This is of importance in unraveling the mechanisms underlying kidney damage. Even with the small number of patients available, and within the limits of study design and study methodology, we were able to demonstrate statistically significant effects suggesting that Cushing's disease may contribute, directly or indirectly, to a reduction in GFR.

Keeping these limitations in mind, our findings might have important clinical implications. Four of our patients with Cushing's disease (22%) showed a GFR below 60 ml/min per 1.73 m² at the time of examination. According to the National Kidney Foundation guidelines (18), these patients should be re-examined (i.e. to confirm that GFR is still <60 ml/min per 1.73 m²) and have additional evaluation by analysis of blood and urine and by imaging studies of the kidneys. They should be treated for their comorbid conditions and evaluated and treated for complications. Patients with an estimated GFR between 60 and 89 ml/min per 1.73 m², with additional cardiovascular risk factors should be further evaluated for markers of renal damage; this means proteinuria, other abnormalities in urine sedimentation tests and detection of renal pathology using imaging tests. Measures need to be taken to slow the progression of renal disease, and there is a clear need for risk evaluation and interventions related to cardiovascular risk factors and treatment of concomitant factors.

In conclusion, to our knowledge our pilot study is the first to report a decreased GFR in patients with Cushing's disease. In addition, we found that disease duration is the strongest predictor for GFR. Consequently, an aggressive initial treatment of patients with Cushing's disease should be considered. Our findings should also be taken into account when designing follow-up strategies for patients with Cushing's disease. Close follow-up of patients with Cushing's disease (even if they are cured), early detection and strict control of cardiovascular risk factors and monitoring of GFR at regular intervals seems mandatory for patients with Cushing's disease. Furthermore, for certain drugs, the dosage should be adapted to the individuals GFR.

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