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ORIGINAL PAPER

Impairment of flow-mediated vasodilatation of brachial artery in patients with Cushing's Syndrome

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Abstract
Background Cushing's Syndrome (CS) is associated with excess and premature cardiovascular disease. Endothelial dysfunction is the initiating event in the development of atherosclerosis. Endothelial function is assessed by flow-mediated dilatation (FMD) of brachial artery. The aim of this study was to assess FMD in patients with CS.
Methods We prospectively evaluated 22 patients with CS (12 women, 10 men; aged 42 ± 11 years, serum cortisol 28.2 ± 14 μg/dl, 24-h urinary free cortisol (UFC) 269 ± 92 μg/day), and 23 control subjects (13 women, 10 men; aged 43 ± 10 years, serum cortisol 14 ± 4 μg/dl, 24 h cortisol 60 ± 22 μg/day). Endothelial function, measured as FMD of the brachial artery using ultrasound, was calculated in two groups. Endothelial function was evaluated by assessing 1-min postischemic FMD of the brachial artery.
Results FMD was lower in patients with CS than that in those without (11.7 ± 4.8% vs. 15.8 ± 3.2%, $P = 0.0001$, respectively). There was no significant difference between two groups regarding baseline diameter of brachial artery. But, hyperemia diameter was lower in patients with CS than without CS (3.6 ± 0.22 mm vs. 3.9 ± 0.19 mm, $P = 0.04$, respectively)
Conclusion Endothelium-dependent FMD may impair in patients with CS compared to controls. Measurement of endothelial function may identify high-risk individuals early and therapy to reduce or retard endothelial dysfunction in patients with CS may lead to decreased cardiovascular morbidity and mortality.

Keywords Cushing's syndrome · Flow-mediated dilatation

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Introduction

Cushing's Syndrome (CS) is associated with increased mortality and morbidity due to cardiovascular disease. Myocardial infarction, heart failure, and stroke in patients with CS may cause a mortality rate four times higher than that expected in the normal population matched for sex and age [1–3]. CS is associated with hypertension due to an increase of glucocorticoids, changes in sodium/volume homeostasis, vascular responsiveness, disturbances in the renin-angiotensin-aldosteron system, and reduced activity of vasodilator system, including prostacyclin, kinin-kallikrein, and nitric oxide synthase [4, 5].

Endothelial dysfunction is an early phase of atherosclerosis [6] and can be measured non-invasively using high-resolution ultrasonography to measure post-ischemic flow-mediated dilatation (FMD) of conduit arteries [7]. Impaired FMD is an early marker of atherosclerotic degeneration and has been shown to be correlated with coronary endothelial dysfunction [8].

The incidence of early atherosclerosis and hypertension in CS-patients is high, which may explain the high mortality from cardiovascular disease, including myocardial infarction, stroke, and aortic rupture [9]. Glucocorticoids may potentiate coronary risk factors, such as hypertension, dyslipidemia, insulin resistance, hyperinsulinemia, or

impaired glucose tolerance. The catabolic effect of hypercorticolism promotes endothelial damage and increases vascular permeability [4, 10].

However, it is not clear whether endothelial function is impaired in patients with CS. To our knowledge, FMD of brachial artery in the patients with CS has not yet been investigated. The aim of this study was to investigate FMD of the brachial artery in patients with CS and controls matched for age, gender, body mass index, smoking, blood pressure, lipid levels, diabetes mellitus, hypertension and duration of hypertension.

Methods

Patients

In the present study, 30 patients (16 women, 14 men; aged 41 ± 10 years) were evaluated. Eight patients were excluded for having coronary heart disease ($n = 2$), moderate-severe valvular heart disease ($n = 2$), chronic renal failure ($n = 1$), or atrial fibrillation ($n = 3$). The remaining 22 patients with CS (12 women, 10 men; aged 42 ± 11 years) were compared with 23 control cases (13 women, 10 men; aged 43 ± 10 years), purposely matched for age, gender, body mass index, smoking, blood pressure and lipid levels, diabetes mellitus, hypertension and duration of hypertension. A total of 12 patients had a confirmed diagnosis of pituitary-dependent CS (Cushing's Disease) (6 macro-and 6 microadenomas), nine had cortisol-secreting adrenal adenomas, and one had cortisol-secreting adrenal carcinoma.

The diagnosis of CS was based on the clinical signs and symptoms and hormonal data. The diagnosis of hypercortisolism was based on increased daily urinary free cortisol (UFC) excretion, rise in serum cortisol concentrations at 8.00 a.m., with absence of physiological circadian rhythm or failure of cortisol to suppress after standard 2-day low-dose (2 mg/day) dexamethasone suppression test (LDDST) (cut-off 1.8 μg/dl). ACTH levels, standard 2-day, high dose (8 mg/day) DST (HDDST), corticotropin-releasing hormone (CRH) stimulation test, and appropriate imaging (pituitary MRI, adrenal MRI/computed tomography (CT) scan were used for the differential diagnosis of CS. CD was diagnosed in the presence of normal/elevated ACTH levels, suppression >50% of serum cortisol levels after HDDST and/or ACTH response >35% to the CRH test. Adrenal CS was diagnosed in the presence of suppressed ACTH levels, lack of suppression of serum cortisol levels after HDDST and/or lack of response of ACTH to the CRH stimulation test. All patients with CD underwent surgical selective resection of the ACTH-secreting pituitary adenomas by the transsphenoidal approach; immunohistochemistry confirmed the diagnosis in all patients. All adrenal adenomas were surgically removed. The diagnosis was additionally confirmed by histological examination. In one of the patients with adrenal CS, radiological characteristics of the mass (size, infiltration, enhancement, dishomogeneity) with hepatic metastases suggested malignancy. The patients postoperatively received glucocorticoid replacement therapy (prednisolone, 5–7.5 mg/day) for a period of 6–24 months until pituitary and adrenal functions returned to normal.

The patients had no bundle branch block, paced rhythm, atrioventricular block, restrictive, hypertrophic or dilate cardiomyopathies, congenital heart disease, hyperthyroidism, or hypothyroidism. We included patients with no history of angina pectoris, myocardial infarction, or congestive heart failure. In addition, the patients had no complaints or physical signs of congestive heart failure or coronary heart disease. Electrocardiography showed sinus rhythm without any signs of ischemia in all of the patients.

Flow-Mediated Dilatation

A standard protocol was used to assess endothelial function, as previously reported, according to recommendation [11]. Brachial arter diameter was measured from B-mode ultrasound images using a 7.5 MHz linear array transducer (Hewlett-Packard Co.). For the FMD of brachial artery, patients fasted ≥ 8 h before the study. Patients were studied in a quiet, temperature-controlled room, at 9:30 a.m. Caffeine intake and cigarette smoking were prohibited for at least 4–6 h before the study. The right arm was immobilized using two cushions supporting the elbow and the wrist. A sphygmomanometric cuff was placed on the forearm. After 10–15 min of rest, the brachial artery was visualized longitudinally with the ultrasonic scanner operating B mode. After an optimal image of the artery was obtained, the ultrasonic transducer was fixed in this position with a custom-built probe holder. Brachial arter diameter was determined in end-diastole, indicated by the R wave of the electrocardiogram. After three baseline measurements were obtained, ischemia was induced by the inflation of the cuff to 100 mm Hg greater than the systolic arteriel pressure to occlude arterial flow for 5 min. After the deflation of the cuff, diameter measurements were performed 30 s, 1 min, 2 min, 3 min, and 4 min, consecutively. Since the arterial dilatation most-likely related to nitric oxide release occurs at 1-min after ischemia, we used FMD at 1 min postischemia to represent the spontaneous endothelial function. Maximal obtained diameter during ischemia-induced hyperemia was used for the calculation of the percentage of FMD (maximum diameter-baseline diameter)/baseline diameter × 100. Endothelial function study was performed by two experienced operators.

The intraobserver and interobserver reproducibility of resting arterial diameter were 0.01 ± 0.01 mm and 0.01 ± 0.02 mm, respectively.

Biochemical Assays

Blood was collected in the morning between 08:00 and 09:00 A.M after an overnight fast to avoid the differences of diurnal variation, especially for hormonal parameters. Serum cortisol levels were measured by automated chemiluminescence method (Dpci Immulate). The determination of 24-h excretion of UFC was assayed by chemiluminescence method (Dpci Immulate). Serum ACTH levels were measured by automated immunoradiometric assay (IRMA) (Immulate 2000 DPC, Diagnostic Product Corporation, 5210 Pacific Concourse, Los Angeles, USA). Normal ranges of biochemical parameters were 6.2–19.4 μg/dl for serum cortisol, 20–90 μg/day for 24-h UFC levels, 9–52 pg/ml for serum ACTH.

Statistical analysis: All the results are expressed as mean ± standard deviations. Baseline and echocardiographic variables were compared by Chi-square test for categoric variables and Student *t*-test for continuous variables. Pearson and Spearman correlation coefficients were used for calculation. A value of $P < 0.05$ was considered statistically significant.

Results

The baseline characteristics of the patients and control subjects are listed in Table 1. There were no significant differences between CS-patients and controls regarding age, gender, smoking, dyslipidemia, body mass index, diabetes mellitus, fasting glucose levels, hypertension, duration of hypertension, and antihypertensive treatment.

Results of ultrasound measurements in the brachial artery are summarized in Table 2. FMD was lower in patients with CS than that in those without (11.7 ± 4.8% vs. 15.8 ± 3.2%, $P = 0.0001$, respectively) (Fig. 1). There was no significant difference between two groups regarding baseline diameter of brachial artery. But, hyperemia diameter was lower in patients with CS than those without CS (3.6 ± 0.22 mm vs. 3.9 ± 0.19 mm, $P = 0.04$, respectively)

No correlation was observed between the cortisol levels and FMD.

Discussion

To the best of our knowledge, this study is the first to investigate FMD of the brachial artery in patients with CS. In the present study, we evaluated FMD of the brachial artery in CS-patients. The control groups matched to CS-patients for age, sex, smoking, hypertension, diabetes mellitus, and pharmacological treatments were selected. We found that FMD of the brachial artery was reduced in patients with CS.

Impairment of endothelium-dependent vasodilatation is an early phenomenon of atherogenesis and it is present before the anatomic evidence of atherosclerosis [12, 13]. Impaired FMD is an early marker of atherosclerotic degeneration and has been shown to be correlated with extent and severity of coronary artery disease [14]. Endothelial dysfunction can be measured non-invasively by ultrasound and Doppler techniques to detect postischemic FMD of the brachial artery [11]. Conduit artery endothelial dysfunction is associated with abnormal vasomotor responses of the coronary circulation [14, 15]. Endothelial function is impaired in the systemic arteries of asymptomatic patients with dyslipidemia, smokers, and coronary artery disease [16, 17].

The precise mechanism of endothelial dysfunction in CS is not well understood. Morphologic and functional alterations of vascular smooth muscle cells may lead to impaired vasoreactivity of the brachial artery. Cortisol is thought to have a range of effects on cardiovascular function [17, 18]. The catabolic effect of hypercortisolism may promote endothelial damage and increases vascular permeability. Tissue effect of cortisol could also include potentiation of cardiac angiotensin II and noradrenaline responsiveness or stimulation of the local renin-angiotensin system [19–21]. In addition, inhibition of vasodilator systems, including nitric oxide, kinin/kallikrein or prostacyclin and inhibition of peripheral catabolism of catecholamine, in particular of noradrenaline, may affect endothelial function.

In CS, vascular system may be affected presenting of atherosclerotic plaques. Recently, Kirilov et al. [22] showed that an increased endothelin-1, a potent vasoconstrictor with hypertensive, mitogenic, and atherogenic effects, were found in patients with CS. Recent studies showed that vascular endothelial growth factor, a highly specific chemotactic and mitogenic factor for vascular endothelial cells, may be involved in the development and stabilization of atherosclerotic plaques by inducing neoangiogenesis [23]. Zacharieva et al. [24] found that vascular endothelial growth factor levels were higher in patients with CS than the patients of essential hypertension.

Smoking and patients who had received any medication such as angiotensin converting enzyme inhibitor, angiotensin II receptor blocker, calcium channel blocker, beta blocker, or statin may affect endothelial function [25–27]. But, in our study, there were no significant differences regarding calcium channel blocker and beta blocker

Table 1 Clinical and laboratory characteristics of study population

	Patients (n = 22)	Controls (n = 23)	P
Age (years)	42 ± 11	43 ± 10	NS
Sex (women/men)	12/10	13/10	NS
Body mass index (kg/m^2)	28 ± 2.3	27 ± 2.7	NS
SBP/DBP (mmHg)	151 ± 2/96 ± 3	150 ± 3/95 ± 2	NS
Pulse rate (beat/minute)	86 ± 7	84 ± 6	NS
Hypertensive/normotensive patients	10/12	9/14	NS
Duration of hypertension (months)	11.5 ± 11	11.8 ± 12	NS
Diabetes mellitus	6	5	NS
Fasting serum glucose (mg/dl)	86 ± 7	92 ± 9	NS
Total cholesterol (mg/dl)	206 ± 35	205 ± 32	NS
Triglycerides (mg/dl)	162 ± 80	165 ± 90	NS
HDL-C (mg/dl)	49 ± 10	48 ± 10	NS
LDL-C (mg/dl)	147 ± 31	148 ± 38	NS
Serum cortisol (μg/dl)	28.2 ± 14	14 ± 4	0.0001
24-h UFC (μg/24-h)	269 ± 92	60 ± 22	0.0001
Beta-blocker (%)	4 (18)	4 (17)	NS
Calcium channel blocker (%)	5 (22)	5 (21)	NS

NS, non significant ($P > 0.05$); SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; UFC, urinary free cortisol

Table 2 Results of ultrasound measurements in the brachial artery

	Patients (n = 22)	Controls (n = 23)	P
Baseline diameter (mm)	3.2 ± 0.42	3.3 ± 0.59	NS
Hyperemia diameter (mm)	3.6 ± 0.22	3.9 ± 0.19	0.04
FMD (%)	11.7 ± 4.8	15.8 ± 3.2	0.0001

FMD, flow-mediated dilatation; NS, $P > 0.05$

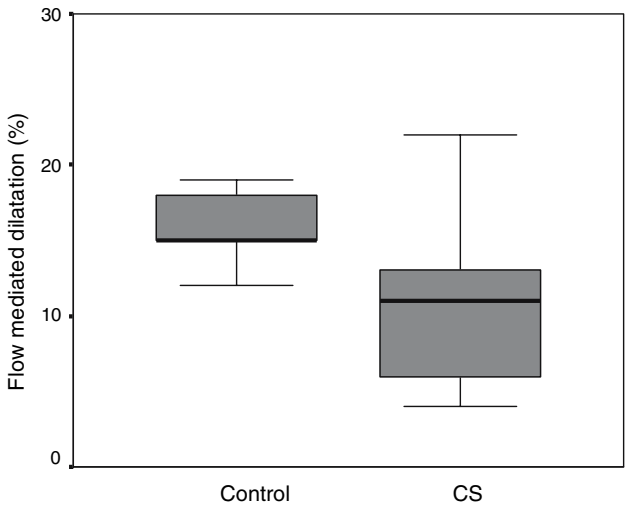

Fig. 1 Flow-mediated dilatation (FMD) in 22 patients with Cushing's Syndrome (CS) and 23 control groups (P = 0.0001)

treatment between the groups. The patients and control subjects did not received angiotensin converting enzyme inhibitor, angiotensin II receptor blocker, or statin therapy.

Limitations

First, hypertension and diabetes mellitus may affect LV function. However, we made an effort by matching patients with CS blood pressure values, duration of hypertension and antihypertensive therapy. In our study, there was no significant difference regarding the hypertension, antihypertensive treatment, or diabetes mellitus. In all patients diabetes was well controlled at the time of study by oral hypoglycemic agents, and mean fasting glucose levels were similar among the groups.

Second, the existence of coronary heart disease cannot be ruled out, because non-invasive stress test or angiography were not performed. However, there was no clinical, electrocardiographic, or echocardiographic ischemic evidence.

Last, this study involves a small number of patients. Therefore, large prospective studies are needed to establish FMD of brachial artery in patients with CS. In addition, lack of assessment of nitrate-induced vasodilatation remains as a limitation of our study.

In conclusion, the results of this study indicate that endothelial dysfunction may develop in the preclinical phase of vascular disease in patients with CS. Measurement of endothelial function of the brachial artery could be useful to

identfy a high-risk of CS-patients. Strategies to reduce or retard endothelial dysfunction in patients with CS may lead to decreased cardiovascular morbidity and mortality.

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