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ORIGINAL ARTICLE

Ambulatory blood pressure monitoring-derived short-term blood pressure variability is increased in Cushing's syndrome

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Abstract Cushing's syndrome is associated with high cardiovascular morbility and mortality. Blood pressure (BP) variability within a 24-h period is increasingly recognized as an independent predictor of cardiovascular risk. The aim of our study was to investigate the short-term BP variability indices in Cushing's syndrome. Twenty-five patients with Cushing's syndrome (mean age 49 ± 13 years, 4 males; 21 Cushing's disease and 4 adrenal adenoma patients) underwent 24-h ambulatory BP monitoring (ABPM) and evaluation of cardiovascular risk factors. Cushing patients were divided into 8 normotensive (NOR-CUSH) and 17 hypertensive (HYP-CUSH) patients and were compared with 20 normotensive (NOR-CTR) and 20 hypertensive (HYP-CTR) age-, sex-, and BMI-matched control subjects. Short-term BP variability was derived from ABPM and calculated as the following: (1) standard deviation (SD) of 24-h, daytime, and nighttime BP; (2) 24-h weighted SD of BP; and (3) average real variability (ARV), i.e., the average of the absolute differences between consecutive BP measurements over 24 h. In comparison with controls, patients with Cushing's syndrome, either normotensive or hypertensive, had higher 24-h and daytime SD of BP, as well as higher 24-h weighted SD and ARV of BP ($P = 0.03$ to $P < 0.0001$). No difference in metabolic parameters was observed between NOR-CTR and NOR-CUSH or between HYP-CTR and HYP-CUSH subgroups. ABPM-derived short-term BP variability is increased in Cushing's syndrome, independent of BP elevation. It may represent an additional cardiovascular risk factor in this disease. The role of excess cortisol in BP variability has to be further clarified.

Keywords Blood pressure · Hypertension · Cushing's syndrome

Introduction

Cushing's syndrome is the result of chronic cortisol overproduction. Patients with endogenous hypercortisolism, including those having subclinical presentation, show a cluster of systemic manifestations, including abdominal adiposity, arterial hypertension and cardiovascular abnormalities, impaired glucose tolerance/diabetes, dyslipidemia, and hypercoagulability [1–4]. All these features are associated with increased cardiovascular risk, which may persist even after normalization of cortisol levels and is the main cause of mortality [3, 5–8]. Multiple factors contribute to the pathogenesis of cardiovascular damage, but the common biological link is cortisol overproduction. High blood pressure (BP) is indeed a common feature of Cushing's syndrome, being present in 70–80 % of adults and in 50 % of children and adolescent patients, and may

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be the cause of the initial consultation [9, 10]. In addition, the circadian blood pressure rhythm measured by 24-h ambulatory BP monitoring (ABPM) has been found altered in patients with Cushing's syndrome, with a characteristic non-dipping pattern, independent of BP levels [11]. In this regard, it is known that either hypertensive or normotensive non-dipper individuals tend to have greater end-organ damage and increased cardiovascular mortality [12–14]. The degree of short-term (i.e., within a 24-h period) BP variability and newer ABPM-derived BP variability indices [15–17] have been more recently recognized as independent predictors of cardiovascular risk, either in hypertensive cohorts or in general populations [18–21].

The aim of our study was to investigate the ABPM-derived short-term BP variability indices in a cohort of patients with Cushing's syndrome.

Materials and methods

Study population

We studied 25 consecutive patients with newly diagnosed Cushing's syndrome. All patients had signs or symptoms specific to overt hypercortisolism, such as moon face, striae rubrae, skin atrophy, easy bruising, proximal muscle weakness. All patients had been evaluated in an outpatient endocrine clinic of the Department of Medicine at the University of Padova in the past 6 years. The diagnosis was based on standard criteria and proved at surgery in all cases [3]. The mean age was 49 ± 13 years (range 22–68 years). There were 21 females and 4 males. The initial diagnosis was based on clinical features, high 24-h urinary free cortisol (UFC) (range 390–3,200 nmol/day, normal 55–330 nmol/day), loss of circadian rhythm of serum cortisol, and failure to suppress with low-dose dexamethasone. The differential diagnosis included high-dose dexamethasone and corticotropin-releasing hormone tests, and ACTH measurements. Morphological assessment was performed by pituitary and adrenal computerized tomography and/or magnetic resonance imaging, and adrenal scintiscan. When indicated, bilateral petrosal sinus sampling was also employed. There were 21 cases of Cushing's disease and four cases of Cushing's syndrome as a result of a cortisol-secreting adrenal adenoma. Disease duration ranged from 3 to 25 months and was estimated as an approximation from the time of onset of clinical hypercortisolism. All Cushing patients were studied while they were awaiting corrective pituitary or adrenal surgery, and no patient had been previously treated with medical therapy specifically aimed to inhibit ACTH or cortisol secretion.

According to the 2013 ESH–ESC Guidelines for the management of arterial hypertension [22], on the basis of 24-h ABPM-derived BP values, Cushing patients were divided into two subgroups: 8 normotensive patients (NOR-CUSH) and 17 hypertensive (i.e., with mean 24-h BP ≥ 130 and/or ≥80 mmHg) patients (HYP-CUSH). The two patient subgroups were compared with 20 normotensive (NOR-CTR) and with 20 essential hypertensive controls (HYP-CTR), respectively. Among Cushing patients with an adrenal adenoma, one patient pertained to the NOR-CUSH subgroup and three patients pertained to the HYP-CUSH subgroup. Normotensive and hypertensive control subjects were randomly recruited from the general population and from a larger essential hypertensive population (more than 500 patients), respectively, seen at our clinics in the same time period. The NOR-CTR and HYP-CTR subgroups were matched for age, sex, and body mass index (BMI) with NOR-CUSH and HYP-CUSH subgroups, respectively. No normotensive control patient had ever received chronic treatment with glucocorticoids or drugs known to affect glucose or lipid metabolism. None had liver or kidney dysfunction. All hypertensive patients, either Cushing or essential hypertensive controls, underwent ABPM after a short period (10–15 days) of antihypertensive treatment withdrawal or were never treated before the study. Six patients with Cushing's syndrome (2 NOR-CUSH and 4 HYP-CUSH) and 4 control patients with essential hypertension had diabetes mellitus and were on oral glucose-lowering drugs at the time of the study.

Informed consent was obtained in all cases. The study was approved by the local ethical committee.

Ambulatory blood pressure monitoring

All patients underwent 24-h ABPM (Takeda TM2430, Asahi, Japan), which was carried out in a day separate from the follow-up office visit. Recording started between 8:30 and 9:00 a.m. Patients were instructed to closely report daily activities during the recordings. Recording was programmed for every 15 min over a 24-h period. Measured values of systolic BP (SBP), diastolic BP (DBP), and heart rate were stored in a digital memory. The readings of the automatic recorder were checked against those obtained with a mercury sphygmomanometer at the beginning and at the end of each 24-h monitoring session; a difference within ±5 mm Hg was considered an adequate agreement between the two methods. Measured values of systolic blood pressure, diastolic blood pressure, and heart rate were downloaded from the monitor to an IBM PC (IBM Co., New York, NY, USA) using custom software. Blood pressure and heart rate measurements were excluded from

the analysis when they were missing or labeled as technically erroneous by the monitor software. If a blood pressure recording had less than 70 % successful readings, it was rejected and repeated. During the monitoring, a written diary of physical and mental activities as well as sleep duration was kept. Blood pressure readings of patients were discarded in case of a lack of sleep during the night (patients not included in the study). Daytime and nighttime periods used for ABPM were from 8:00 a.m. to 10:00 p.m. and from midnight to 6:00 a.m., respectively. Blood pressure dipping was defined as a night–day systolic and diastolic fall ≥10 % [23].

Short-term blood pressure variability measures

Short-term BP variability was derived from ABPM and calculated as the following: (1) standard deviation (SD) of 24-h, daytime, and nighttime BP [17]; (2) the average of daytime and nighttime SD, each weighted for the duration of the day and night periods (24-h "weighted" SD of BP), which allows to remove the mathematical interference from nighttime BP fall [17]; and (3) average real variability (ARV), i.e., the average of the absolute differences between consecutive BP measurements over 24 h [16].

Clinical and laboratory methods

From all individuals, clinical parameters and blood samples for biochemical parameters were obtained in the morning after overnight fasting, at the time of ABPM study. Twenty-four-hour UFC was measured by radioimmunoassay using a kit from Diagnostic Products Co. (CA, USA). The intra-assay coefficient of variation (CV) was 6 %, and the inter-assay CV was 8.2 %. The normal range was 55–330 nmol/day. Plasma ACTH was measured by chemiluminescence (Immulite 2000, Diagnostic Products Co., CA, USA): intra-assay CV 6.2 % and inter-assay CV 4.8 %; normal range at 8:00 a.m. 2–11 pmol/L. Serum cortisol was measured by chemiluminescence (Immulite 2000, Diagnostic Products Co., CA, USA): intra-assay CV 7.4 % and inter-assay CV 5.2 %; normal range at 8:00 a.m. 138–690 nmol/L. All other biochemical variables were assayed in plasma or serum using standard methods.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS-PC version 20.0; SPSS Inc., Chicago, Illinois, USA). All results are expressed as mean ± SD for continuous variables and as proportion for categorical variables. Continuous data were subjected to the Kolmogorov–Smirnov test to determine their distribution. Differences between means were assessed by Student's *t* test or by Mann–Whitney *U* test in non-normally distributed data for two-sample comparison, or by one-way analysis of variance (ANOVA) applying Fisher's least significant difference (LSD) post hoc test for multiple comparisons. Chi-square statistics was used to assess differences between categorical variables. Relationships between continuous variables were assessed by calculating the Pearson's correlation coefficient or the Spearman's rank correlation coefficient, when appropriate. P values < 0.05 were taken as statistically significant.

Results

All Cushing patients were studied during disease activity, and their mean urinary cortisol level was 1,082.2 ± 549.9 nmol/day, significantly higher than in controls (196.1 ± 76.2 nmol/day, $P = 0.0001$). Age, sex, and BMI were similar (P = NS) in the entire Cushing (49 ± 13 years, 4 males and 21 females, 27.5 ± 5.2 kg/m^2, respectively) and in the entire control group (48 ± 12 years, 5 males and 35 females, 26.7 ± 5.4 kg/m^2, respectively). Twenty-four-hour SBP/DBP and heart rate did not differ between the entire Cushing population and the entire control population (137.6 ± 13.3/84.6 ± 9.7 vs 132.2 ± 12.9/81.4 ± 9.5 mmHg, and 78.7 ± 9.1 vs 75.5 ± 8.5 beats per minute, respectively, P = NS). The proportion of Cushing patients with a non-dipping BP profile (10/25, i.e., 40 %) at ABPM was higher than the proportion (9/40, i.e., 22.5 %) of hypertensive controls ($P < 0.001$). No differences in biochemical parameters were observed between the entire Cushing group and the entire control group.

Table 1 summarizes clinical and biochemical parameters, i.e., fasting blood glucose, total cholesterol and triglycerides, in the Cushing and control subgroups. Level of glycemic control, hypercholesterolemia, i.e., total cholesterol ≥ 5.2 mmol/L, hypertriglyceridemia, i.e., triglycerides ≥ 1.7 mmol/L, and obesity (BMI ≥ 30 kg/m^2) were equally distributed in both Cushing and essential hypertensive patients with diabetes mellitus. No difference in biochemical parameters was observed between NOR-CTR and NOR-CUSH patients or between HYP-CTR and HYP-CUSH subgroups.

Table 2 summarizes 24-h ABPM values and ABPM-derived short-term BP variability parameters in the different Cushing and control subgroups. Mean 24-h as well as daytime and nighttime SBP/DBP did not differ between NOR-CTR and NOR-CUSH or between HYP-CTR and HYP-CUSH subgroups. In comparison with controls, patients with Cushing's syndrome, either normotensive or hypertensive, had higher SD of 24-h and daytime SBP/DBP as well as higher weighted SD and ARV of 24-h SBP/

Table 1 Clinical and biochemical differences between normotensive Cushing patients (NOR-CUSH), hypertensive Cushing patients (HYP-CUSH), normotensive controls (NOR-CTR), and essential hypertensive controls (HYP-CTR)

	NOR-CTR	HYP-CTR	NOR-CUSH	HYP-CUSH	*P* values
N	20	20	8	17	P_{NOR} = NS
					P_{HYP} = NS
Age	47 ± 10	50 ± 14	47 ± 13	50 ± 12	P_{NOR} = NS
					P_{HYP} = NS
Gender (male/female)	3/17	5/15	1/7	3/14	P_{NOR} = NS
					P_{HYP} = NS
BMI (kg/m^2)	24.7 ± 4.9	28.7 ± 5.2	28.1 ± 5.9	27.2 ± 4.8	P_{NOR} = NS
					P_{HYP} = NS
Clinic systolic BP (mmHg)	131.0 ± 10.8	160.5 ± 13.9	130.0 ± 7.0	163.2 ± 17.8	P_{NOR} = NS
					P_{HYP} = NS
Clinic diastolic BP (mmHg)	82.5 ± 7.4	101.0 ± 9.8	81.8 ± 3.4	98.2 ± 6.2	P_{NOR} = NS
					P_{HYP} = NS
Clinic heart rate (beats/minute)	78.3 ± 13.4	77.0 ± 10.5	74.5 ± 5.2	77.7 ± 7.5	P_{NOR} = NS
					P_{HYP} = NS
Fasting blood glucose (mmol/L)	4.9 ± 0.3	5.1 ± 0.4	5.4 ± 1.2	5.3 ± 1.2	P_{NOR} = NS
					P_{HYP} = NS
Total cholesterol (mmol/L)	5.1 ± 1.0	5.2 ± 1.1	5.1 ± 0.8	5.3 ± 1.2	P_{NOR} = NS
					P_{HYP} = NS
Triglycerides (mmol/L)	1.0 ± 0.5	1.3 ± 0.5	1.2 ± 0.4	1.1 ± 0.4	P_{NOR} = NS
					P_{HYP} = NS
UFC (nmol/day)	196.6 ± 74.6	209.5 ± 75.4	961.7 ± 270.2	1,139.0 ± 632.6	$P_{NOR} < 0.0001$
					$P_{HYP} < 0.0001$

Mean values ± SD

P_{NOR} = NOR-CTR vs NOR-CUSH; P_{HYP} = HYP-CTR vs HYP-CUSH

BMI body mass index; *BP* blood pressure; *UFC* urinary free cortisol

DBP ($P = 0.03$ to $P < 0.0001$). No difference for nighttime SD of SBP/DBP between NOR-CTR and NOR-CUSH subgroups, and for nighttime SD of SBP/DBP between HYP-CTR and HYP-CUSH subgroups was observed. There was no difference between NOR-CUSH and HYP-CUSH patients in UFC levels and in the prevalence of diabetes, hyperlipidemia, and obesity. All levels of statistical significance for short-term BP variability parameters were maintained, even though the 3 HYP-CUSH patients and the one NOR-CUSH patient with an adrenal adenoma were not considered for analysis. No correlation between 24-h UFC levels and the different 24-h BP variability measures was found in patients with Cushing's syndrome.

Discussion

Ambulatory BP monitoring has enabled a non-invasive estimate of BP variability to be obtained. Several studies suggest that ABPM-derived BP short-term variability can have a prognostic relevance, predicting organ damage and cardiovascular events over and above the contribution provided by average BP values in different populations [18–21]. The main finding of our report is that ABPM-derived short-term BP variability is increased in patients with Cushing's syndrome, either hypertensive or normotensive, in comparison with corresponding controls. Among different parameters, the differences in the weighted 24-h SD BP variability between our patient groups appear rather small. This index is not influenced by nocturnal BP fall, which is commonly absent in Cushing's syndrome, and seems to correlate better with end-organ damage than conventional 24-h SD of BP [17]. However, the relevance of this approach with respect to cardiovascular outcomes when populations are compared needs to be assessed more extensively.

Generally, the mechanisms hypothesized to explain increased BP variability in the short term include [20, 21] (1) central and reflex autonomic modulation (i.e., an increased central sympathetic drive and impaired baroreflex sensitivity); (2) elastic properties of arteries (i.e., a reduced arterial compliance); (3) dysfunction of rheological (i.e., increased blood viscosity) and humoral (insulin, angiotensin II, bradykinin, endothelin-1, nitric oxide)

Table 2 Ambulatory blood pressure differences between normotensive Cushing patients (NOR-CUSH), hypertensive Cushing patients (HYP-CUSH), normotensive controls (NOR-CTR), and hypertensive controls (HYP-CTR)

	NOR-CTR	HYP-CTR	NOR-CUSH	HYP-CUSH	*P* values
24-h Systolic BP (mmHg)	121.5 ± 7.5	143.0 ± 7.0	123.4 ± 10.6	144.3 ± 8.2	P_{NOR} = NS
					P_{HYP} = NS
24-h Diastolic BP (mmHg)	75.2 ± 4.6	87.5 ± 9.2	77.1 ± 6.8	88.1 ± 8.8	P_{NOR} = NS
					P_{HYP} = NS
24-h SD of systolic BP (mmHg)	12.2 ± 3.4	14.7 ± 4.0	17.2 ± 3.2	18.0 ± 5.6	P_{NOR} = 0.008
					P_{HYP} = 0.03
Daytime SD of systolic BP (mmHg)	10.0 ± 2.1	12.9 ± 3.8	17.5 ± 3.6	16.9 ± 5.1	P_{NOR} < 0.0001
					P_{HYP} = 0.003
Nighttime SD of systolic BP (mmHg)	8.0 ± 2.3	10.6 ± 4.1	9.9 ± 3.0	15.1 ± 13.4	P_{NOR} = NS
					P_{HYP} = NS
24-h SD of diastolic BP (mmHg)	10.5 ± 2.0	11.7 ± 2.5	14.6 ± 4.1	15.4 ± 4.6	P_{NOR} < 0.0001
					P_{HYP} = 0.002
Daytime SD of diastolic BP (mmHg)	8.3 ± 1.8	9.8 ± 2.6	14.8 ± 4.5	15.2 ± 4.9	P_{NOR} < 0.0001
					P_{HYP} < 0.0001
Nighttime SD of diastolic BP (mmHg)	6.8 ± 1.4	8.6 ± 2.7	7.3 ± 3.5	11.3 ± 8.2	P_{NOR} = NS
					P_{HYP} = NS
24-h Weighted SD of systolic BP (mmHg)	9.4 ± 1.4	12.2 ± 3.0	15.2 ± 3.2	16.4 ± 6.3	P_{NOR} < 0.001
					P_{HYP} = 0.004
24-h Weighted SD of diastolic BP (mmHg)	7.9 ± 1.3	9.4 ± 2.0	12.5 ± 4.1	14.1 ± 4.7	P_{NOR} = 0.001
					P_{HYP} < 0.0001
24-h ARV of systolic BP (mmHg)	7.5 ± 1.3	9.3 ± 2.1	14.4 ± 5.4	14.1 ± 4.8	P_{NOR} < 0.0001
					P_{HYP} = 0.001
24 h ARV of diastolic BP (mmHg)	6.7 ± 1.5	7.7 ± 1.4	12.1 ± 4.3	12.3 ± 4.2	P_{NOR} = 0.01
					P_{HYP} = 0.004

Mean values ± SD

P_{NOR} = NOR-CTR vs NOR-CUSH; P_{HYP} = HYP-CTR vs HYP-CUSH

BP blood pressure; *SD* standard deviation; *ARV* average real variability

factors; and (4) emotional (i.e., psychological stress) and behavioral factors. Indeed, all these aspects should be discussed to explain the increased BP variability in our Cushing patients.

Several studies refer to the importance of sympathetic activity and arterial baroreflexes in regulating cardiovascular variability and report other factors, including the vascular response to sympathetic stimuli, playing a role in determining the strength of BP oscillations [24, 25]. As far as the autonomic nervous system activity is concerned, direct recording of muscle sympathetic activity, pharmacologic autonomic blockade, reflex function tests, plasma catecholamine concentrations, and noradrenaline spillover data all showed that sympathetic activity is not increased in glucocorticoid excess states [26, 27]. This seems to be against a primary central action being responsible for increased BP variability observed at daytime in our Cushing patients, either normotensive or hypertensive. However, it has been reported that sensitivity to catecholamines/angiotensin II or other vasoactive molecules is higher in Cushing's syndrome [26]. Thus, fluctuations in circulating factors having larger vascular effect and leading to higher daytime BP variability cannot be excluded in our Cushing patients. ABPM-derived nighttime BP variability parameters were similar in NOR-CTR and NOR-CUSH, as well in HYP-CTR and HYP-CUSH subgroups. Based on disparity between ABPM-derived daytime and nighttime BP variability, the arterial baroreflex failure does not fully explain our data. We have previously shown in Cushing's patients an overall (daytime and nighttime) diminished heart rate variability [28], and this finding was recently confirmed by others [29]. Therefore, it is possible that mechanisms modulating BP variability, baroreflex activity, and heart rate variability are differently involved at nighttime in Cushing's syndrome.

Regarding reduced arterial compliance, a series of clinical investigations have documented in Cushing' syndrome an increased carotid artery stiffness [30–32]. However, more data measured by appropriate methodology on the morphology and function of different vascular body

districts, including aortic stiffness, are needed [33]. Among rheological factors, only one of our Cushing patients had polycythemia (i.e., hematocrit 51.9 %) and no correlation was found between hematocrit and the different ABPM-derived short-term BP variability indices in the whole Cushing group (data not shown). Cushing's syndrome is also accompanied by an increased risk of arterial thrombosis due to hypercoagulable state [34]; however, none of the arterial (including coronary) thrombotic events was observed in the clinical history of our Cushing patients. An insulin-resistant state and/or diabetes has been found to be associated with high ABPM-derived short-term BP variability [21, 35]; in this respect, our data cannot exclude in some of our Cushing patients a role of previously altered blood glucose levels, in spite of an apparent drug-related glycemic control at the time of the study. Again, it is known that active Cushing's syndrome is associated with a high prevalence of psychopathology aspects, including mood and anxiety disorders [36]. Blood pressure response to mental stress is reported to be normal in Cushing's syndrome [26]. However, the potential impact of a higher level of persistent emotional distress on BP has never been systematically explored in this disease.

Finally, we propose a novel pathogenic mechanism involving a direct biological link between cortisol overproduction and increased ABPM-derived short-term BP variability in Cushing's syndrome. In the setting of 24-h cortisol secretory profile, an abnormally increased amplitude and frequency of serum cortisol secretory bursts have been shown in patients with Cushing's disease, restored by successful surgery [37, 38]. It is known that cortisol can selectively target the vascular wall, acting on specific receptors located on vascular smooth muscle cells and endothelial cells [39, 40], and can locally suppress the production of vasodilators such as prostacyclin and nitric oxide [41, 42]. Higher circulating cortisol fluctuations in patients with Cushing's syndrome might lead to rapidly increased vascular wall contraction, inducing simultaneous higher BP fluctuations. The hypothesis of a direct vascular effect of cortisol is further supported in our study by the occurrence of a similarly increased BP variability in both normotensive and hypertensive Cushing subgroups. No statistical relationship was found between 24-h BP variability indices and 24-h UFC levels in our patients with endogenous cortisol excess. This may be not surprising, since the coincidence between single circulating cortisol peaks and BP oscillations was not specifically tested. Other possible limitations of the study are the influence of gender on the results, due to overwhelming prevalence of females, and the relatively small size of patient groups, which could have led to a statistical type 2 error.

In conclusion, ABPM-derived short-term BP variability is increased in patients with Cushing's syndrome, independent of BP elevation. It may represent an additional cardiovascular risk factor in this disease. The role of excess cortisol in BP variability has to be further clarified.

Conflict of interest The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported. This research did not receive any specific grant from any funding agency in the public commercial or non-profit section.

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