

Plasma 11 β ,17 α ,21-Trihydroxypregn-4-ene-3,20-dione as Blood Biomarker Assay

A controlled study

EDWIN SEVER BECHIR¹, MIHAELA JANA TUCULINA^{2*}, MARA CARSONTE³, IONELA TEODORA DASCALU², MIHAELA RAESCU⁴,
ANDREEA NICOLA², CRISTIAN NIKY CUMPAIA⁴

¹ Medicine, Pharmacy, Science and Technology University of Tirgu Mures, Faculty of Dental Medicine, 38 Gheorghe Marinescu Str., 540142, Tirgu Mures, Romania

² Medicine and Pharmacy University of Craiova, Faculty of Dental Medicine, 2-4 Petru Rares Str., 200349, Craiova, Romania

³ Carol Davila University of Medicine and Pharmacy, C.I. Parhon National Institute of Endocrinology, 34-38 Aviatorilor Av., Bucharest, Romania

⁴ Titu Maiorescu University of Bucharest, Faculty of Dental Medicine, 67A Gheorghe Petrascu Str., 031593, Bucharest, Romania

11 β ,17 α ,21-Trihydroxypregn-4-ene-3,20-dione or cortisol (C₂₁H₃₀O₅) represents the product of adrenal glands and in humans its assessment is a useful tool for evaluation of glucocorticoid axes. Our purpose is to evaluate the profile of morning plasma cortisol/ACTH/ionogram in menopausal women with non-functioning adrenal tumours. A controlled study of 193 menopausal women found that BMI is statistically significant higher versus control in subjects with unilateral, respective bilateral adrenal non-secretor tumours. Baseline C₂₁H₃₀O₅ is similar between the groups while ACTH is decreased when compare with control group indicating a potential persistent cortisol tumour-related exposure. Glycated haemoglobin A1c is increased in group with single adrenal mass (versus control) while serum sodium is higher in group with double adrenal masses (versus control).

Keywords: cortisol, blood, biomarker, menopause, adrenal, sodium

11 β ,17 α ,21-Trihydroxypregn-4-ene-3,20-dione or cortisol (C₂₁H₃₀O₅, molecular mass of 362.460 g/mol) represents the product of adrenal glands and in humans its assessment represents a useful tool for evaluation of glucocorticoid axes [1-3]. The biomarker is available in blood, but also in 24-hours urine (urinary free cortisol) and saliva [3-5]. Hair cortisol have a potential role but its use in common practice is still limited [6,7]. As hormone, C₂₁H₃₀O₅ is included in steroid class of hormones of glucocorticoid subtype [1-3]. The physiological origin of the molecule is in fasciculate area of adrenal glands-included cortical part while the synthetic analogues like hydrocortisone and others derivatives are widely used in multiple heterogeneous areas of medicine [8]. The role of human 11 β ,17 α ,21-Trihydroxypregn-4-ene-3,20-dione as an endocrine gland-derived product is reflected in blood glucose control, immune defence, fat and proteins metabolism, blood pressure maintenance, neuromodulation, collateral loop in bone formation - resorption balance, and adjustment of stress response especially in long term [9,10]. Also, the glucocorticoid axis influences other endocrine systems like non-ACTH (Adrenocorticotrophic Hormone) pituitary hormones, gonadal axes, and bone regulation [11-14]. The use of cortisol assay in daily endocrine practice is related to the differentiation with reactive (functional) hypercorticism, and pseudo Cushing syndromes (as seen in obesity, depression, chronic alcoholism), and to the evaluation of endogenous Cushing syndrome forms [15-18].

Our purpose is to evaluate the profile of morning plasma cortisol/ACTH/ionogram assays in menopausal women with non-functioning adrenal tumours.

Experimental part

Material and method

Study design: This is a transversal controlled study in Romanian population. Three groups of study were analysed: with bilateral adrenal tumour (group A), with unilateral adrenal tumours (group B) and also a group of subjects without adrenal tumour who were considered control group or asymptomatic (healthy) group in terms of potential adrenal hyper-secretion (group C).

Inclusion criteria

The subjects included in the study were adult female in menopause (independent of the menopausal type: surgical or spontaneous). All the patients agreed to anonymously use their medical records at the moment of their clinical evaluation during admission on endocrinology department. The patients group A and B were asymptomatic from a clinical point of view regarding the potential secretion of cortical and medullar adrenal gland. The evaluation for group A and B was done by endocrinologist in order to establish the diagnosis of non-functioning adrenal tumour (incidentaloma) according to current guidelines and by imagist to assess the presence of unilateral/bilateral tumour based on abdominal computed tomography [19,20]. All the patients enrolled in group A and B were classified as non-secretor pattern of the tumour based on dexamethasone suppression test (1 milligram or 2 days of 2 milligrams per day) with a second day level of morning plasma cortisol suppressed under 1.8 μ g/dL.

* email: mtuculina@yahoo.com

All the authors equally contributed to the drawing up of the present paper

Exclusion criteria: The patients who were under treatment with glucocorticoid therapy were not included. Neither was the persons taking corticoids through any route during the last 6 months. We did not include males and pre-menopausal women. Also the patients with active cancers of any kind, and those with active secretor endocrine tumours of any site were not enrolled.

The assessment: At anamnesis age, age at menopause (and calculated period of time since menopause), the presence of high blood pressure, dyslipidaemia of any kind, type 2 diabetes mellitus was registered. Body Mass Index (BMI). The plasma morning cortisol and ACTH (Adrenocorticotrophic Hormone) were assessed through a venous puncture. Also, chemical blood parameters like glycated haemoglobin and ionogram (serum sodium and potassium) were evaluated. The chemical parameters are introduced as mean, median, standard deviation (SD), minimum and maximum values. Statistical significance cut off was $p < 0.05$.

Results and discussions

The groups' clinical parameters: 193 females were included: 9 in group A, 21 in group B and 163 in control group (Fig. 1). The average age was between 57 and 62 years, and the mean period of time in menopause is between 14 and 16 years (table 1). When compare each two groups, they were similar as age (years) and period of time in menopause (years), except for p-value between group B and C regarding age (table 2). Further adjustment for age was used in analysis. Mean BMI was not different between the groups with one or two tumours (table 3). Control group had statistical significant lower BMI versus women with tumours of group A, respective group B (table 3). Mean BMI corresponds to obesity in group A, to overweight for group B and C (table 3). The percent of subjects with arterial hypertension was in each group of 88.88% (group A), 76.66% (group B), 53.84% (group C); hyperlipemia: 88.88% (group A), 56.66% (group B), 61.53% (group C); type 2 diabetes mellitus and impaired glucose tolerance: 11.11% (group A), 30% (group B), 19.01% (group C).

The groups' chemical non-endocrine parameters: The patients with unilateral adrenal tumours had statistical significant higher glycated haemoglobin (HbA1c) versus the subjects of control group. The mean value of HbA1c was abnormal only in group B (table 4). The level of serum sodium was statistically significant increased when compare the women with bilateral tumours to control. The maximum value of sodium exceeded upper normal limit of normal in group A. Serum potassium was similar between the groups (table 4).

The groups' chemical endocrine parameters: Morning plasma cortisol as baseline assay was not statistically significant different between the three groups. ACTH was

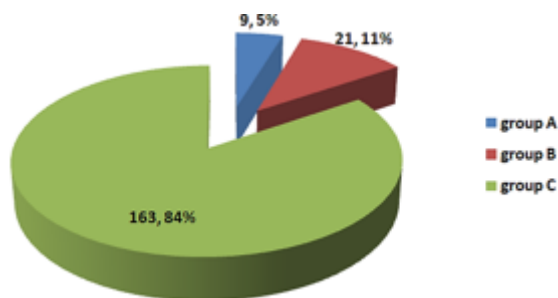


Fig. 1. The groups included in the study: A -menopausal patients with bilateral adrenal tumours, B -subjects with unilateral non-functioning tumour, C -control group (N=193 patients)

Table 1

THE A,B,C GROUPS OF MENOPAUSAL FEMALES: AGE AND YEARS SINCE MENOPAUSE

parameter	age	menopausal period of time
Units	years	years
	group A	
mean	60.222	15
median	58	13
min	45	3
max	77	32
SD	11.02	10.267
	group B	11----43
mean	57.636	14.206
median	57	13
min	42	0
max	79	35
SD	9.296	8.178
	group C	44----194
mean	62.158	16.034
median	62	14
min	45	1
max	84	44
SD	8.369	9.892

Table 2

STUDENT TEST BETWEEN THE THREE GROUPS REGARDING AGE AND YEARS SINCE MENOPAUSE

	age	menopausal period of time
p-value A-B	0.48	0.8
p-value B-C	0.006	0.3
p-value A-C	0.5	0.7

Table 3

BMI ANALYSIS IN STUDIED GROUPS

BMI	group A
mean	30.612
median	25
min	24
max	50
SD	9.281
	group B
mean	29.244
median	28.07
min	18
max	55
SD	6.782
	group C
mean	26.686
median	25
min	17
max	45
SD	5.601
student ttest (p-value)	
A-B	0.6
B-C	0.02
A-C	0.05

statistically significant lower in group A versus C, respective group B versus C (table 5).

No statistical significant correlation between cortisol, respective ACTH and either of the following parameters: age, years since menopause, and BMI was established.

Observation: The minim values of ACTH were suppressed in a few cases for group A and B but those cases were included because the dexamethasone suppression test indicated an adequate (normal) level of less than 1.8 $\mu\text{g/dL}$ of second day morning plasma cortisol.

Limits of the study: In this pilot study in Romanian menopausal women we did not quantify the level of daily stress from a psychological point of view, neither the presence of depression and current exposure to antidepressants since they may influence the glucocorticoid axes assessment. This might influence the

Table 4

GLYCATED HAEMOGLOBIN IN % (HbA1c, NORMAL 4.5-5.9%), SODIUM IN MMOL/L (NORMAL LEVELS 135-145 mmol/L) and POTASSIUM IN mmol/L (NORMAL VALUES BETWEEN 3.5 AND 5 mmol/L) ANALYSIS IN STUDIED GROUPS

parameter	HbA1c	sodium	potassium
group	group A		
mean	5.75	143.2	4.536
median	5.75	144	4.635
min	5.4	139	3.9
max	6.1	147	5.1
SD	0.494	2.949	0.402
group	group B		
mean	7.3975	140.379	4.508
median	6.95	141	4.5
min	5.99	129	3.67
max	9.7	145	5.22
SD	1.6	3.509	0.397
group	group C		
mean	5.731	140.486	4.369
median	5.6	141	4.31
min	0.055	134	3.64
max	10.1	145	5.81
SD	1.1759	2.45	0.416
student ttest (p-value)			
p-value A-B	0.24	0.1	0.8
p-value B-C	0.009	0.8	0.1
p-value A-C	0.9	0.02	0.34

level of baseline morning plasma cortisol. In term of blood ionogram, we did not measure the influence of anti-hypertensive medication. Also, the dexamethasone suppression test was not routinely done to all the patients in control group.

Conclusions

In our controlled study of 193 menopausal women, BMI is statistically significant higher versus control in subjects with unilateral, respective bilateral adrenal non-secretor tumours. Morning plasma $11\beta,17\alpha,21$ -Trihydroxypregn-4-ene-3,20-dione is similar between the groups while morning plasma ACTH is decreased when compare with control group indicating a potential persistent cortisol tumour-related exposure. Glycated haemoglobin A1c is increased in group B versus C while serum sodium is higher in group A versus C.

Abbreviations

ACTH = Adrenocorticotrophic Hormone

BMI = Body Mass Index

HbA1c = glycated haemoglobin

SD = standard deviation

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Table 5

MORNING PLASMA CORTISOL AND ACTH (ADRENOCORTICOTROPIC HORMONE) IN THREE GROUPS OF THE STUDY (NORMAL VALUES FOR CORTISOL ARE 6-21 μ G/DL, FOR ACTH 3-66 pg/mL)

parameter	cortisol	ACTH
Units	microg/dL	pg/mL
group	A	A
mean	14.216	8.559
median	14.46	5.919
min	9.73	1.07
max	17.92	20.78
SD	3.341	7.948
group	B	B
mean	15.35	13.61
median	15	9.13
min	6.22	1
max	35.82	58.55
SD	7.971	13.469
group	C	C
mean	15.484	22.104
median	12.955	19.04
min	5.7	9.67
max	22	44
SD	11.046	8.627
student ttest (p-value)		
p-value A-B	0.7	0.4
p-value B-C	0.9	0.005
p-value A-C	0.8	0.002

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