ECRONICON OPEN ACCESS

EC ENDOCRINOLOGY AND METABOLIC RESEARCH
Research Article

Assessment of Prediction of Relapse of ACTH-Dependent Cushing's Syndrome in Patients Undergoing Transsphenoidal Surgery

Z Yu Halimova[1,2] and U A Mirsaidova[1]*

[1]Republican Specialized Scientific and Practical Medical Center of Endocrinology of the Ministry of Health of the Republic of Uzbekistan Named After Acad. Y.H. Turakulov, Department of Neuroendocrinology with Pituitary Surgery, Tashkent, Uzbekistan

[2]Endocrinology with Pediatric Endocrinology Unit, Tashkent Pediatric Medical Institute, Tashkent, Uzbekistan

***Corresponding Author**: U A Mirsaidova, Republican Specialized Scientific and Practical Medical Center of Endocrinology of the Ministry of Health of the Republic of Uzbekistan Named After Acad. Y.H. Turakulov, Department of Neuroendocrinology with Pituitary Surgery, Tashkent, Uzbekistan.

Received: May 06, 2020; **Published:** May 28, 2020

Abstract

Objective: To analyse the utility of a test with desmopressin to assess the effectiveness of transsphenoidal surgery (TSS) in patients with Cushing's disease (CD) in the post-operative period.

Design: A prospective study of 42 patients with CD operated in 2012 - 2019 was conducted. Patients were divided into 2 groups: I group-18 patients, postoperative basal cortisol ≤ 138 nmol/L, remission group and IIgr-24 patients with cortisol levels ≥ 138 nmol/L, a risk group for recurrence of the disease, although they had clinical and biochemical remission. All patients underwent a standard test with desmopressin (Newell-Price J, 1997) with a cortisol and ACTH level of 0', 30', 60', 90', 120'.

Results: The use of TD in the studied groups revealed an increased (hyper reaction) in 16.6% of patients of group I of cortisol in 2 times (on average 280.02 ± 12 nmol/L) and ACTH in 1.4 times (average 14.02 ± 2.06 ng/dl) compared with other patients, the results of which were between 30' and 60' achieved to 166.2 ± 34.4 nmol/L and 15.39 ± 3.4, respectively, which is lower than in the healthy control in 3.5 and 1.5 times with levels of 485.9 ± 25 nmol/l and 23.5 ± 1.79 ng/dl. In group II, 25% (6 patients) also had an increased response to TD between 30' and 60', while the average data in this group were significantly higher by 1.5 times and 6.6 times than group I ($p < 0.05$, $p < 0.01$) and lower by 2.6 times and 4.3 times higher than in the control ($p < 0.001$, $p < 0.005$).

Conclusion: A complete indicators using modern methods of statistical processing gave us to identify TD as the most sensitive test in determining remission and shows the insufficiency of only determining ACTH and cortisol in this.

Keywords: *Cushing's Disease; Surgery; Desmopressin; Relapse*

Introduction

However, even when patients appear to be remission, a recurrence rate of CD is observed up to 3% in the first year and 10 - 20% in patients followed up for sufficiently long periods. Therefore, early intervention is crucial for the prevention of the complications of hypercortisolaemia in patients in seeming remission [13,26,34]. However, there are controversy in the criteria used for the diagnosis of initial remission [5,13,25] and this is clearly important to assess the risk of the relapse after surgery. This variety of criteria of assessment a remission also creates the challenges to compare a treatment results in various studies [12-15,33]. According to the most authors, a post-operative morning (09.00h) serum cortisol level in the first 7 days after surgery is the most important marker in assessment of remission after TSS [2,4,5,28,39], using a threshold of equal to or less than 138 nmol/L (< 5 μg/dl) [5,13], or more strictly as < 1.8 μg/dl (50 nmol/l) [18]. Nevertheless, in some cases cortisol level might fall a gradually during long period of time [6,20,23]. There are not enough evidence on the prognostic value of the postoperative serum ACTH level, which might to predict a remission of the disease.

According to several studies [1,4,17,36], a level of serum cortisol less then < 50 nmol/L after surgery indicates a persistent remission and a low risk of relapse (not more than 10% in 10 years). If there were variation between 50 - 140 nmol/l, it recommended to closely

monitor patients and a serum cortisol level of more than 140 nmol/L an indicator of high risk of relapse (20 - 30%). Examination of urinary free cortisol (UFC) is more problematic as normative values vary and the risk of relapse are assay-dependent [2,38,40].

To better predict an apparent remission and the risk of relapse, several studies have investigated the use of desmopressin as a post-operative predictor of later recurrence. The hypothesis is that even the post-operative serum cortisol appears to be low, tumorous corticotrophs, unlike normal ACTH-secreting cells, will retain an enhanced response to this V2-receptor agonist. Thus, a response will an indicate the presence of residual tumor and an increased probability of relapse. Various studies using slightly different protocols have suggested an absent cortisol response to the desmopressin test (DT), which had a negative prognostic value (NPV) of 76% - 100%, with a sensitivity to predict relapse from 20% to 100% and specificity from 57% to 100%. Some of this variation may be contingent on using percentage responses to the DT rather than absolute changes. It should be said that in 4 separate studies [18,38,43,44], which used the same threshold values of cortisol (7 - 7.4 μg/dl; 193 - 204 nmol/l), in a total 116 patients, the negative predictive value of the DT was 92% with a specificity of 95% with a positive predictive value of 77% and a sensitivity of 68% in predicting later relapse.

This research has hypothesized whether use of DT might predict a relapse to compare with 09:00h serum cortisol level in patients with CD after TSS with the main focus in Central Asia.

Materials and Methods

We have investigated the use of the DT in comparison to an 09.00h post-operative serum cortisol in 42 patients with confirmed CD and performed surgery in the neurosurgery departments of the RSSPMC of Endocrinology in the period from 2000 to 2019. All studies were approved by the Ethics Committee of the Ministry of Health (No. 7/50-1210 of 08/08/2019) and all patients signed informed consent. Inclusion criteria were patients who underwent TSS for CD by TSS.

The diagnosis of CD was based on presenting clinical features, failure to suppress 09.00h serum cortisol on a low-dose dexamethasone suppression test (DST), suppression on a high-dose DST, a significant abnormality on MRI scanning (see below) and positive post-operative histopathology of a corticotroph adenoma.

Exclusion criteria were patients with ACTH-independent Cushing's syndrome, patients with the ectopic Cushing syndrome and patients who were in pharmacotherapy, post-X-ray irradiation, combination therapy or adrenalectomy.

Before surgery, all patients underwent pituitary MRI with a contrast agent with gadolinium using a Magnetom Trio A Tim 1.5 Tesla apparatus (SIEMENS, Germany). The size of the adenoma was evaluated according to the largest diameter. It is established that the pituitary microadenoma is adenoma less than 10 mm in diameter while macroadenoma 10 mm or more. Invasive growth was assessed by classifying the degree of invasion of pituitary adenomas into the cavity of the cavernous sinus according to Knosp Scale. Assessment of the results of MRI was performed by a specialist neuroradiologist.

Post-operative procedure

Following TSS, the 42 patients were divided into two groups depending on the level of post-operative serum cortisol according to the Clinical Practical Recommendations of the Society of Endocrinologists (2015). The desmopressin test was performed in the morning between 08.00 and 09.00 hours after an overnight fast. Desmopressin tests were performed on 5^{th} - 6^{th} day in postoperatively period. All the hormonal replacement therapy with steroids (cortisone acetate) was stopped the day before postoperative testing. Group I (18 patients) was defined as patients with a basal blood cortisol level of ≤ 138 nmol/L who were allocated as remission achieved, while group II (24 patents) were patients with a basal blood cortisol level ≥ 138 nmol/L. The control group consisted of 20 healthy individuals (10 men and 10 women) in the same age.

Assays

Serum cortisol was determined by radioimmunoassay (RIA) (BekhmanCoulter, Czech Republic using Gamma-12 and Strantg 300 counters). The reference values are: morning (08.00 - 9.00h, 260 -720 nmol/L, evening (23.00-00.00h, <50 mmol/L. UFC was determined by the method of radioimmunoassay (RIA) (BekhmanCoulter, Czech Republic on counters Gamma-12 and Strantg 300), with reference values 38-208 nmol/24h. Plasma ACTH was determined by RIA (BekhmanCoulter, Czech Republic on counters Gamma-12 and Strantg 300), with reference values less than 50 ng/ml.

Sampling

Blood for plasma ACTH and serum cortisol were collected in fasting at 08.00 - 9.00h and then at 23.00-00.00h (awake); for plasma ACTH the sample was rapidly centrifuged, then snap frozen. UFC was collected over 24h in the standard manner.

The desmopressin test

For the DT, desmopressin acetate 4 μg from (Ferring Pharmaceuticals Ltd, UK) was used after approval of the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan No. 7/50-1210 (dated 08.08.2019).The test was carried by following steps: After an 8-hour overnight fast, in the morning at 09.00h, an indwelling catheter was placed in an antecubital vein with the patient supine for 120 minutes and during the entire study period. At 09.00h, 8 ml of blood (0 minute) were taken and 10 μg of desmopressin was administered as an intravenous bolus injection. Further, blood samples for ACTH and cortisol were obtained after 30, 60, 90 and 120 minutes. Blood pressure and heart rate were recorded throughout the study period. In order to avoid possible fluid overload and hyponatraemia, have been recommended to restrict a fluid intake (not more than 1.5 - 2 liters) for the rest of the day.

Statistics

Statistical analysis of the results were performed by the software package StatSoftStatistica 6.1, IBM SPSS Statistics 20.0. Quantitative data are represented by central trends and scatter arithmetic mean (M) and standard deviation (SD) in the format M (± SD). A comparison of two independent groups with a normal distribution was carried out using Student's t-test. In this case, using other criteria, the null hypothesis was rejected at $p \leq 0.05$.

Results

42 patients after TSS were examined. 71.4% (30 patients) were women, 28.6% were men (12 patients). The average age of women was 32.4 ± 3.1SD years (range 15 - 49 years), men - 32.0 ± 1.75SD (range 26 -39) years. The duration of the disease from the moment of diagnosis was 41.6 ± 9.2 months (from 7 to 131 months).

The first group of patients consisted of 18 patients (42.8%) with cortisol levels were ≤ 138 nmol/L in the first 5 - 7 days after TSS (group I). From these, 12 were women (70%), 6-men (30%). The average age of women was 36.75 ± 5.87 years (from 20 to 49 years), men - 31.5 ± 0.35 years (from 23 to 36 years). The duration of the disease from the moment of established diagnosis was 34.6 ± 13.4 months (from 7 to 96 months), the age of the patients at the time of diagnosis was on average 29.33 ± 4.26 years, 32.25 ± 6.35 years for women and 23.5 ± 0.35 years in men. The duration of the preoperative period of the disease was within 32.3 ± 3.81 months (from 19 to 53 months), the duration of the postoperative period was 29.83 ± 10.79 months (from 8 to 80 months).

As shown in table 1, serum cortisol and plasma ACTH at 09.00h and UFC were all significantly higher in Group I compared to Group II and both Groups showed higher values in all 3 parameters compared to the control group.

Parameters	1 gr. (n = 18)		2 gr. (n = 24)		Control (n = 20)	P-value
Sex	F = 12	M = 6	F = 18	M = 6		
Age	36,7 ± 5,9	31,5 ± 0,3	29,5 ± 3,0	32,5 ± 4,6	31,85 ± 1,91	
Age at time of diagnosis (years)	29,33 ± 4,26		24,37 ± 2,34			$p_1 < 0,1$
Duration. preoperative period (month)	32,3 ± 3,81		53,75 ± 5,28			$P_1 < 0,1$
Postoperativeduration period (month)	29,83 ± 10,79		20,75 ± 4,57			$P_1 < 0,5$
ACTH before surgery (ng/dl)	59,1 ± 2,39		76 ± 6,6		21,7 ± 1,66	$p_1 < 0,1$ $p_2 < 0,001$ $p_3 < 0,05$ $p_4 < 0,005$ (1 -group) $p_4 < 0,05$ (2-group)
ACTH after (ng/dl)	18,6 ± 4,73		35,86 ± 12,75			$p_1 < 0,5$
UFC before (nmol/l)	177,1 ± 18,18		305,13 ± 62,3		154,15 ± 4,21	$p_1 < 0,5$ $p_2 < 0,1$ $p_3 < 0,01$ $p_4 < 0,001$(1 –group) $p_4 < 0,1$(2-group)
UFC after(nmol/l)	26,9 ± 4,49		119 ± 35,96			$p_1 < 0,05$
Basal cortisol before (nmol/l)	848,8 ± 35,18		968,58 ± 98,72		46,8 ± 24,52	$p_1 < 0,5$ $p_2 < 0,001$ $p_3 < 0,01$ $p_4 < 0,001$(1 –group) $p_4 < 0,05$(2-group)
Basal cortisol after (nmol/l)	75,2 ± 8,78		412,87 ± 55,7			$p_1 < 0,05$

Table 1: *Clinical and hormonal characteristics of patients in the studied groups (n = 62).*
Note: P: Significance of differences, P1: Significance of differences between group I and II, P2: I and control, P3: II group and control, P4: Before and after surgery.

A serum cortisol during 09.00h in patients of Group I showed a fall in mean cortisol to 75.2 ± 8.78 nmol/L (range from 41 - 132 nmol/l), plasma ACTH (mean 18.6 ± 4.73 ng/dl, range 6.5 - 43.1 ng/dl) and UFC (26.9 ± 4.49 nmol/L (range from 16 - 57 nmol/L) after surgery.

It should be noted that in 3 patients (16%), despite the achievement of biochemical remission, the clinical signs of the disease did not regress. The objective status of patients was characterized by a stability of BMI of 45.7 kg/cm^2 and resistance to blood pressure. A detailed

analysis of these cases showed that the patients had an average age of 32.2 years, an average disease duration of 108 months and a pre-operative period of 36 months. In general, these patients did not differ from patients in this group, with the exception of a 3-fold increase in the average duration of the disease.

In Group II, after a TSS a serum blood cortisol levels were ≥ 138 nmol/L, where 75% (18 patients) were women, 25% (6 patients) were men. The average age of women was 29.5 ± 3.0 years (15 - 45 years old), men - 32.5 ± 4.59 years (15 - 45 years old). The duration of the disease from the moment of diagnosis was 46.87 ± 11.97 months (from 8 to 131 months). The age of patients at the time of diagnosis was 24.37 ± 2.34 years old, including 23.3 ± 2.99 years old for women and 27.5 ± 3.18 years old for men.

The duration of preoperative period was 53.75 ± 5.28 months, and the postoperative period was 20.75 ± 4.57 months. Preoperative levels of ACTH were - 76 ± 6.6 ng/dL, UFC 305.13 ± 62.23 nmol/L and basal cortisol level was 968.58 ± 98.72 nmol/L, which are 3.5 fold ($p \leq 0.05$), 1.98 fold and 2 fold ($p \leq 0.01$) higher respectively compared with the control group.

In Group II, serum blood cortisol level after a TSS was ≥ 138 nmol/L, mean basal cortisol was 412.87 ± 55.7 (range 177 - 900 nmol/L), plasma ACTH 35.86 ± 12.75 ng/dl (range 5.2 - 177 ng/dl), and UFC119 ± 35.96 nmol/L (23 - 364 nmol/L).

Despite the fact that remission was not achieved by the levels of postoperative basal cortisol, clinical remission was observed in 6 patients in group II. Regression of symptoms like disproportionate obesity, excessive hair growth, increased blood pressure, weakness in the lower extremities, menstrual irregularities, and amenorrhea, changes in appearance, abdominal striae have been observed. By the analyze the data of these 6 patients separately, it was observed that 66.6% (4 patients) were women, 33.4% (2 patients) were men. The average age of patients was 34.5 ± 1.06 years old. The duration of the disease from the moment of diagnosis was 28 ± 14.14 month (from 8 to 48 months). The age of patients at the time of diagnosis was 29.5 ± 1.06 years old. The duration of the preoperative period was 54 ± 4.24 month postoperative period - 11,02 ± 4,57 months. Preoperative levels of ACTH were 113.35 ± 24.5 ng/dL, UFC 211.5 ± 39.95 nmol/l and basal cortisol 897.8 ± 56.5 nmol/l, i.e. only daily urine free cortisol and basal cortisol was much lower than other patients in this group.

According to the table 1, the average age of women patients in group 2 was significantly less (P1 < 0.1). Moreover, in patients of group II, the disease also manifested much earlier (P1 < 0.1).

The duration of the preoperative period in group 1 was 1.66 times shorter than in patients of group 2 ($p < 0.1$), while the postoperative period was 1.43 times longer ($p < 0.5$). In the preoperative period, hormone levels in patients of group 1 compared with group 2 had the following picture: ACTH was 1.28 times lower ($p < 0.1$), UFC-lower 1.72 times ($p < 0.5$), blood cortisol is 1.14 times lower ($p < 0.5$). That is, in all respects, patients of the second group had significantly high hormone levels initially.

Parameters	1 group	2 group	Control	P-value
Number of patients (n)	18	24	20	-
Early postoperative cortisol (nmol/L)	75,2 ± 8,78	412,87 ± 55,7		$P_1 \leq 0,0005$
Early postoperative ACTH (ng/dl)	18,6 ± 4,73	35,86 ± 12,75		$P_1 < 0,3$
Test desmopressin				
The time of the test with desmopressin after TSS (months)	43,8 ± 10,71	30,3 ± 7,08		$P_1 < 0,3$
Cortisol, 0min, nmol/l	92,71 ± 19,6	103 ± 16,82	466,7 ± 24,55	$P_1 < 0,1$, $P_2 < 0,0005$, $P_3 < 0,0001$
Peak kort60min, nmol/l	136,24 ± 34,4	183,9 ± 26,72	485,94,5 ± 25,55	$P_1 < 0,05$, $P_2 < 0,001$, $P_3 < 0,001$
ΔCort, nmol/l	43,5 ± 16,79	80,83 ± 2,65	19,24 ± 1,044	$P_1 < 0,1$, $P_2 < 0,5$, $P_3 < 0,05$

% ΔCort, nmol/L	36,28 ± 13,53	113,91 ± 3,69	4,11 ± 0,02	$P_1 < 0,1$, $P_2 < 0,5$, $P_3 < 0,05$
ACTH at 0 min, ng/dl	14,13 ± 4,4	53,26 ± 13,3	21,75 ± 1,65	$P_1 < 0,1$, $P_2 < 0,5$, $P_3 < 0,005$
Peak ACTH 30, ng/dl	15,39 ± 4,34	101,66 ± 19,4	23,5 ± 1,79	$P_1 < 0,01$, $P_2 < 0,5$, $P_3 < 0,0005$
Δ ACTH, ng/dl	1,26 ± 0,38	48,4 ± 2,0	1,79 ± 0,13	$P_1 < 0,01$, $P_2 < 0,5$, $P_3 < 0,01$
% Δ ACTH, ng/dl	15,35 ± 4,24	141,6 ± 3,63	8,23 ± 0,01	$P_1 < 0,05$, $P_2 < 0,5$, $P_3 < 0,01$

Table 2: *Desmopressin test results in the study groups (n = 62).*

Note: P: Significance of differences; C: Control, p1: Significance of differences between group I and II, p2: I and control, p3: II group and control, p4: Before and after surgery.

From the data of table 2 it is obvious that the levels of cortisol and ACTH were significantly higher in the group of patients who did not have remission. Cortisol levels were increased between 0' and 30' in the group of patients who did not reach remission and formed a plateau between 30' and 60' minutes, followed by a slow decline. Plasma ACTH levels peaked between 0' and 30' and plummeted between 30' and 60' (Figure 1).

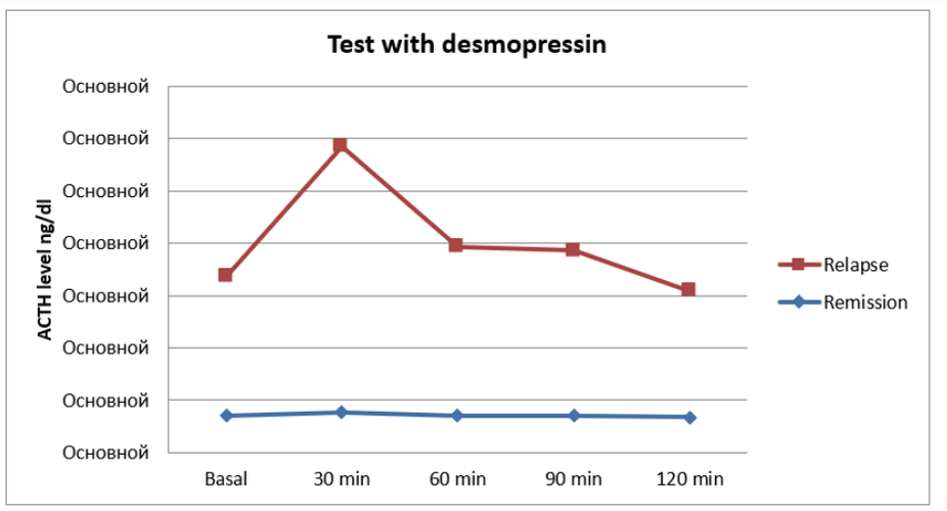

Figure 1: *The results of the dynamics of ACTH in the blood during the test with desmopressin in the study groups. Red line-relapse group and blue line- remission group (changing ACTH levels after desmopressin).*

Desmopressin test results in group 1

The Desmopressin test was performed after TSS for 43.8 ± 10.71 months (from 3 days to 67 months). Thus, early postoperative cortisol in patients of group 1 was recorded within 75.2 ± 8.78 nmol/L and varied from 41 to 132 nmol/L. On the day of the test, the cortisol level at 0' was - 92.71 ± 19.6 nmol/L and was 5 times lower than in the control group ($p \leq 0.0005$) and the peak of the cortisol response was between 30 and 60 min, averaging 136.24 ± 34.4 nmol/L, that is, 3.5 fold lower than in the control group ($p \leq 0.001$), ΔCort 43.5 ± 16.79 nmol/L, cortisol increased on - 36.28 ± 13.53 nmol/l, which is 8.82 fold higher than in the control group ($P \leq 0.05$).

The level of ACTH 0 was 14.13 ± 4.4 ng/dL. The peak of ACTH was in 30 minutes-15.39 ± 4.34 ng/dl, and these indicators were 1.5 fold lower ($p \leq 0.5$) than in control group. Moreover, both Δ ACTH (1.26 ± 0.38 ng/dl) and the increased ACTH (15.35 ± 4.24 ng/dl) were 1.42 and 1.86 fold higher respectively than the control ($p \leq 0,5$).

The results of desmopressin test in group 2.

The desmopressin test have been performed in 24 patients of group 2 after 12 ± 0.7 months (from 3 days to 36 months) of TSS. An early postoperative cortisol level in patients of group 2 was in average 412.87 ± 55.7 mmol/l, varied from 177 to 900 nmol/l. On the day of the test, the average values of basal cortisol in 0 min was 103 ± 16.82 nmol/l, which was 4.53 folder lower than in the control group (from 37.1 to 191.7 nmol/l; $p \leq 0.0001$). The peak of cortisol also occurred between 30 and 60 minutes and constituted 183.9 ± 26.27 nmol/L, which is 2.64 fold lower than in the control group (from 65.2 to 306.3; $p \leq 0.001$). ΔCort (peak cortisol - cortisol 0) 80.83 ± 2.65 nmol/L, which was 4.2 times higher (from 4.2 to 230.6 nmol/L; $p \leq 0.05$), a percentage of cortisol level increased (ΔCort-cortisol 0 * 100%) to 113.91 ± 3.6 nmol/L 9, which was 27.7 fold higher than in the control group ($P \leq 0.05$).

The level of ACTH 0 was 53.26 ± 13.3 ng/dl (2.44 fold higher) than in the control group (from 14.4 to 162.9 ng/dl ($p \leq 0.05$), the peak of ACTH between 30 and 60 minutes was within the range of 101.66 ± 19.4 ng/dl, which was 4.32 fold higher than in the control group (from 24.1 to 243.7 ng/dl; $p \leq 0,0005$), Δ ACTH 48.4 ± 2.0 ng/dl, which was 27 fold higher than in the control group (from 4.2 to 140.7 ng/dl; $p \leq 0.01$), a ACTH increased to 141.6 ± 3, 63, (17.2 fold higher than in the control group) ($P < 0.01$).

A comparative analysis of the desmopressin test results of the studied groups showed that the levels of early postoperative cortisol in patients of group 2 were initially 5.5 fold higher than in the first group ($p \leq 0.005$) and on the day of the test with desmopressin the difference was 1.1 fold higher compared to the first group ($p < 0.5$). With the time, the level of excessed basal cortisol gradually minimized and an average was 92.7 ng/ml in the first and 103 ng/ml in the second ($p < 0.1$) groups. It should be noted that the peak secretion of cortisol against the background of desmopressin in both groups was between 30 and 60 minutes and constituted 136.2 ng/dl and 183.9 ng/dl respectively, with a difference 1.34 fold ($p \leq 0.05$). Δ Cortisol was 1.85 fold higher than the data of the first patients and amounted to 80.8 versus 43.5 ($p \leq 0.1$), the rise of cortisol was lower by 3.13 fold in the first group ($p \leq 0.1$) and the percentage of increase was significantly higher in patients of the 2nd group (113.9% in the second and 36.3% in the first).

A slightly different trend has been observed in changes in the level of ACTH. ACTH levels initially in patients of the 1st group and in the control were within normal range, but in the 2nd group it was significantly higher (3.76 fold) than in the first ($p \leq 0.1$). Peak values of ACTH were observed between 30 and 60 min and it was 6.6 fold higher ($p \leq 0.01$), Δ ACTH 38.4 fold ($p \leq 0.01$) higher, ACTH growth 9.2 fold higher in the second group ($P < 0.05$). ACTH growth coefficient in the first group was 15.35 ± 4.24%, cortisol - 36.28 ± 13.53% versus 141.6% and 113.9% in the second group, respectively.

It should be noted that in the group of patients who were in remission, in 3 patients (16.6%), ACTH levels during TD increased significantly (on average 14.02 ± 2.06 ng/dl), leading to a corresponding increase in cortisol levels (average 280.02 ± 12 nmol/L) in the blood. Moreover, the peak of ACTH secretion was equally high at 30' and 60'. As the test results showed that the average ACTH level and cortisol

level differ in group I from the control group, although not significantly ($p > 0.05$). At the same time, a significant reaction was noted in response to desmopressin, which increased 4 times more than in the control group. The response of cortisol was almost parallel, with the same area under the curve and had no peaks (Figure 1 and 2).

As can be seen from figure 1, in patients of group 1, ACTH levels during the test with desmopressin did not change significantly ($p > 0.05$), while in patients of group 2 at the 30 minutes of the test, there were a significant increase in the level of ACTH in the blood ($p < 0.05$).

As can be seen from figure 2, in patients of group 1, the levels of cortisol during the test with desmopressin did not increase significantly at 30 and 60 minutes ($p > 0.05$), while in patients of group 2 samples of 30 and 60 minutes demonstrated a significant increase in the level of cortisol in the blood ($p < 0.05$). Thus, during the test with desmopressin levels of ACTH and cortisol significantly increased in patients of the 2 groups.

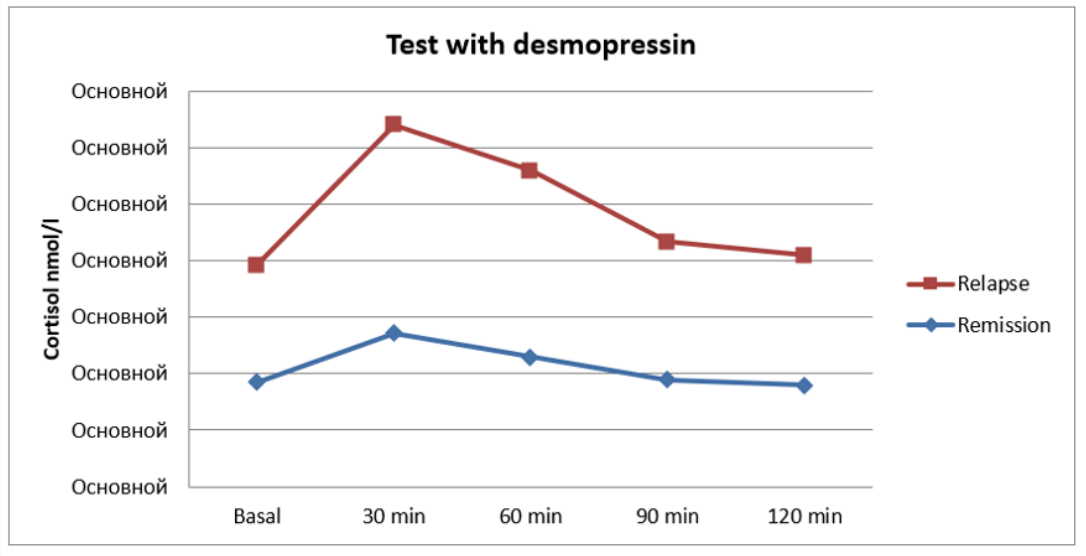

Figure 2: *The results of the dynamics of cortisol in the blood during the test with desmopressin in the study groups. Red line-relapse group and blue line- remission group (changing cortisol levels after desmopressin).*

Thus, an analysis of the data in the studied groups showed that TD is a sensitive tool in determining the reactivity of pituitary corticotrophic cells. Judging by the level of ACTH and cortisol during TD and carefully comparing with clinical, biochemical, hormonal indicators and risk factors, the effectiveness and radicalism of the surgical intervention can be assessed. Therefore, according to the results of our studies, it is clear that TD clearly shows the line of remission and the presence of relapse, as evidenced by the significantly high reactivity of corticotrophs (possibly residual or recurring again) with a sharp overproduction of ACTH and cortisol after their stimulation in patients with uncertain outcomes of the disease or with subclinical cushingoid after TSS. Moreover, TD can help identify cases of a risk group among patients with objective indicators of remission according to the latest clinical recommendations [34]. So, in the cohort of postoperative patients with remission of ACTH-DCS in our study, with a TD, 3 (16.6%) of 18 patients showed an abnormal increase in the level of ACTH and cortisol, i.e. the test was positive, and this indicates the possible risks of tumor growth and relapse of the disease.

In the second group, 25% (6 patients out of 24) of patients had a positive reaction to desmopressin, as evidenced by an increase in cortisol levels, which can serve as a negative prognostic marker of the likelihood of a relapse.

Discussion of the Results of the Study

Currently, the most relevant and debatable issues remain the search for reliable markers of remission ACTH-DCS after TSS. Therefore, there are still debate of about implication of the various tests in the postoperative period - with CRH [8], with dexamethasone, with desmopressin to define whether it can be prognostic tool of relapse in individual cases and for a wide range of patients.

Inviti., *et al.* (1999) showed in 288 adults that only half of patients with clinical remission experienced a decrease in plasma ACTH [24]. Moreover, urine free cortisol (UFC) alone cannot be a measure of remission after TSS. However, multiply assessment of UFC with below the normal in combination with evidence of disease remission in other tests may be useful to confirm the remission of the disease [18]. Most studies in adults showed a prolonged remission if postoperative UFC concentrations are less than 28 - 56 nmol/day (< 10 - 20 μg/day) [16,19,42].

Batista DL., *et al.* (2009) suggested that daily urine free cortisol (UFC) cannot serve as a prognostic factor for long-term remission. The authors investigated the effectiveness of the test with corticotropin-releasing hormone (CRH) in adults after TSS as a predictor of long-term remission and found that 23 adult patients with a reduced response to the test with CRH performed after 6 - 42 months of TSS had no relapse, and 3 relapses occurred in patients (out of 6 in this study) who had a normal response in the test [16]. It was demonstrated that the effectiveness of repeated TSS after relapse varies between 37 - 73% [15,22], and the improvement in results increases in the presence of a localized pituitary adenoma [11,16]. In all cases of postoperative relapse, immunohistochemical confirmation of the diagnosis and/or inferior petrosal sinus sampling (IPSS) with the introduction of a stimulating agent is necessary [6,16].

Lonser R., *et al.* [28] suggested that tumor identification during surgery, the presence of a tumor producing ACTH (as determined by immunohistochemistry), and the presence of a non-invasive tumor of ACTH were positive predictors of initial remission in patients. Moreover, a younger age, a smaller tumor, and the absence of invasion of the cavernous sinus or other dural invasion were associated with prolonged remission. Nevertheless, the minimum morning serum cortisol level < 1 μg/dl after surgery had a positive predictive value of 96% for long-term remission.

According to some authors, when an early postoperative blood cortisol level of less than 50 nmol/L [24,37] or less than 138 nmol/L is reached, it indicates remission; other researchers [27,39] considered similar values of cortisol as a prognostic criterion for relapse of corticotropinoma growth. Thus, there are no strike consensus on this issue. In 2015, the guidelines for the treatment of Cushing's syndrome defined that CD remission should be confirmed if morning cortisol < 5 μg/dL (< 138 nmol/L) or free urine cortisol (UFC) < 10 - 20 μg/24h (< 28 - 56 nmol/24 hours) 7 days after surgery. However, there is also no consensus on the cut-off point of cortisol after surgery (< 5 μg/dl (137.9 nmol/L), < 2 μg/dl (55.2 nmol/L), < 1.8 μg/dl (49.7 nmol/l), < 1.3 μg/dl (35.9 nmol/l) or even < 1 μg/dl (27.6 nmol/l) [34].

According to Colombo P., *et al.* (2000), CD has an uncertain prognosis because patients who have achieved remission after TSS may develop relapse in decades. These authors sought to identify factors predicting relapse by focusing on desmopressin (DDAVP) and corticotropin-releasing hormone (CRH) tests after surgery. After studying 57 patients with CD after TSS, remission was established in 24 cases, late relapse in 15 and persistent diseases in 18 cases. It was found that the average recurrence time was 40 months. ACTH levels increased after stimulation with desmopressin and CRH and a significantly higher response was in the group with late relapse, indicating an increased risk of relapse. In the logistic regression model, an increase in ACTH > 9 pg/ml after TD and > 36.7 pg/ml after CRH showed a sensitivity of 93% and 73%, respectively, the specificity of 82% and 76% in the group with late relapse. The area under the curve was 0.91 for DDAVP, 0.80 for CRH, and 0.95 for DDAVP + CRH, i.e. combined use was more effective than each test individually, but not to a statistically significant degree. The answer to both tests led to a positive predictive value (PPV) of 100%, while none of the tests gave a negative predictive value (NPV) of 100% [18].

According to Le Marc'hadour P., *et al.* (2015), who compared the results of the test with CRH and TD performed both before and after TSS in 24 patients with active Cushing's disease who were monitored for one to 36 months. The researchers found that a significant

ACTH/cortisol response ($P < 0.001$) was induced either by desmopressin. A positive response of cortisol to desmopressin was found in all patients. After pituitary adenectomy, 14 "cured" patients were monitored for 1 - 36 months. The administration of desmopressin did not cause a peak in ACTH or sensitivity to cortisol in any patient. In contrast, a progressive recovery of ACTH and cortisol responses after CRH was observed at different time intervals in all. Five patients whose cortisol concentration normalized only after surgery showed constant sensitivity to desmopressin, and two of them relapsed after 12 and 24 months. In patients who did not achieve remission, hormonal sensitivity to CRH or desmopressin was the same before and after surgery. 10 patients studied only after long-term follow-up, six were cured, and a normal response to CRH was present when changes in ACTH/cortisol concentrations were not caused by desmopressin. The remaining four unsuccessfully operated patients underwent pituitary irradiation, and they showed different and ambiguous hormonal reactions to desmopressin and CRH. The authors concluded that the postoperative observation of patients with Cushing's disease, the maintenance, or disappearance of the hormonal response may be associated with the persistence or complete removal of adenomatous corticotrophs, respectively. It has been suggested that TD should be performed during preoperative assessment and observation of patients with ACTH-dependent Cushing's syndrome [27]. Romanholi., *et al.* (2008) have demonstrated that a large series of patients with CD (107 patients) that the resistance of the ACTH response to desmopressin in the early postoperative period may be associated with a higher risk of late relapse. In the early postoperative period, in patients with CD, TD can stimulate ACTH secretion in a residual corticotrophic tumor, but not in non-tumor cells [39].

Other authors Losa., *et al.* (2009) noted that predicting treatment outcomes for patients with CD is a complex task. Their goal was to evaluate the accuracy of the immediate postoperative plasma cortisol, desmopressin test and dexamethasone-desmopressin combined test (DDCT) as predictors of the result. This retrospective two-center study included 67 patients with initial remission and a minimum postoperative follow-up period of up to 18 months. Follow-up included 3 - 6 months, and then an annual determination of the levels of UFC, ACTH, and cortisol blood with a test of dexamethasone (1 mg) and TD. The authors concluded that the addition of DDCT the first 3 years after surgery to an immediate postoperative evaluation of cortisol allows us to develop an optimal management strategy for patients operated on for Cushing's disease [29].

Losa., *et al.* (2015), have investigated that in 174 patients with CD, after a successful operation, the risk of relapse using TD was revealed. It was found that a positive ACTH response to DDAVP after surgery is associated with an increased risk of CD relapse. However, the specificity and prognostic value of this finding is low [30].

According to our study, in the postoperative period, there was a significant decrease in the hormonal parameters of blood and urine (ACTH, cortisol) compared with preoperative data in patients of both groups with remission and without remission. But at the same time, in the second group, the mean values of ACTH and blood cortisol after the operation were significantly higher, remaining within normal ranges. It should be noted that the early postoperative level of cortisol in patients of group 1 was within 75.2 ± 8.78 nmol/l, and in group 2 it was much higher - 412.87 ± 55.7 nmol/l.

A TD revealed that in all blood samples after surgery in patients of group 2, the levels of ACTH and cortisol were significantly higher than in patients of group 1. Thus, this test are confirmed the severity of the condition of the second group of patients. For accurate answers to questions, further monitoring of the quality of life of patients with ACTH-DSC in the postoperative period is necessary.

Conclusion

A complete anamnesis, clinical, hormonal and visualized analysis of indicators using modern methods of statistical analysis gave us to identify TD as the most sensitive test in determining remission. Thus, evaluating only the level of ACTH and cortisol are insufficient to early prediction of the relapse after TSS.

Bibliography

1. Acebes JJ., *et al*. "Early post-operative ACTH and cortisol as predictors of remission in Cushing's disease". *Acta Neurochirurgica* 149 (2007): 471-479.

2. Alexandraki KI., *et al*. "Long-term remission and recurrence rates in Cushing's disease: predictive factors in a single centre study". *European Journal of Endocrinology* 168 (2013): 639-648.

3. Ambrogio AG., *et al*. "Usefulness of desmopressin testing to predict relapse during long-term follow-up in patients in remission from Cushing's disease". *Endocrine Connections* (2017): 791-799.

4. Aranda G., *et al*. "Long-term remission and recurrence rate in a cohort of Cushing's disease: the need for long-term follow-up". *Pituitary* 18.1 (2015): 142-149.

5. Arnaldi G., *et al*. "Diagnosis and complications of Cushing's syndrome: a consensus statement". *Journal of Clinical Endocrinology and Metabolism* 88 (2003): 5593-5602.

6. Atkinson AB., *et al*. "Long-term remission rates after pituitary surgery for Cushing's disease: the need for long-term surveillance". *Clinical Endocrinology* 63 (2005): 549-559.

7. Atkinson AB., *et al*. "Cyclical Cushing's syndrome first diagnosed after pituitary surgery: a trap for the unwary". *Clinical Endocrinology* 36.3 (1992): 297-299.

8. Avgerinos PC., *et al*. "The corticotropin-releasing hormone test in the postoperative evaluation of patients with cushing's syndrome". *Journal of Clinical Endocrinology and Metabolism* 65.5 (1987): 906-913.

9. Barbot M., *et al*. "Predicting late recurrence in surgically treated patients with Cushing's diseas". *Clinical Endocrinology* 79 (2013): 394-401.

10. Batista DL., *et al*. "Postoperative testing to predict recurrent Cushing disease in children". *Journal of Clinical Endocrinology and Metabolism* 94.8 (2009): 2757-2765.

11. Benvensite RJ., *et al*. "Repeated transsphenoidal surgery to treat recurrent or residual pituitary adenoma". *Neurosurgery* 102 (2005): 10041012.

12. Berker M., *et al*. "Early promising results for the endoscopic surgical treatment of Cushing's disease". *Neurosurgical Review* 37 (2014): 105-114.

13. Biller BM., *et al*. "Treatment of adrenocorticotropin-dependent Cushing's syndrome: a consensus statement". *Journal of Clinical Endocrinology and Metabolism* 93 (2008): 2454-2462.

14. Blevins LS Jr., *et al*. "Outcomes of therapy for Cushing's disease due to adrenocorticotropin-secreting pituitary macroadenomas". *Journal of Clinical Endocrinology and Metabolism* 83 (1998): 63-67.

15. Boscaro M., *et al*. "Cushing's syndrome". *Lancet* 357.9258 (2001): 783-791.

16. Chee GH., *et al*. "Transsphenoidal pituitary surgery in Cushing's disease: can we predict outcome?" *Clinical Endocrinology* 54 (2001): 617-626.

17. Chen JC., *et al*. "Transsphenoidal microsurgical treatment of Cushing disease: postoperative assessment of surgical efficacy by application of an overnight low-dose dexamethasone suppression test". *Neurosurgery* 98 (2003): 967-973.

18. Colombo P., *et al*. "Usefulness of the desmopressin test in the postoperative evaluation of patients with Cushing's disease". *European Journal of Endocrinology* 143 (2000): 227-234.

19. Dickerman RD and Oldfield EH. "Basis of persistent and recurrent Cushing disease: an analysis of findings at repeated pituitary surgery". *Journal of Neurosurgery* 97 (2002): 1343-1349.

20. Esposito F., *et al*. "Clinical review: early morning cortisol levels as a predictor of remission after transsphenoidal surgery for Cushing's disease". *The Journal of Clinical Endocrinology and Metabolism* 91 (2006): 7-13.

21. Estrada J., *et al*. "The complete normalization of the adrenocortical function as the criterion of cure after transsphenoidal surgery for Cushing's disease". *The Journal of Clinical Endocrinology and Metabolism* 86 (2001): 5695-5699.

22. Grigoriev AYu., *et al*. "Repeated transsphenoid adenomectomy in relapse and persistent course of Itsenko-Cushing's disease". *Neurosurgery* 2 (2014): 49-53.

23. Hammer GD., *et al*. "Transsphenoidal microsurgery for Cushing's disease: initial outcome and long-term results". *The Journal of Clinical Endocrinology and Metabolism* 89 (2004): 6348-6357.

24. Invitti C., *et al*. "Glucocorticoid receptors in anorexia nervosa and Cushing's disease". *Biological Psychiatry* 45.11 (1999): 1467-1471.

25. Kirilyuk ML. "Diagnosis and treatment of pituitary Cushing's syndrome". *International Endocrinological Journal* 6.62 (2014): 182-193.

26. Lacroix A., *et al*. "Cushing's syndrome". *Lancet* 386 (2015): 913-927.

27. Le Marc'hadour P., *et al*. "Postoperative follow-up of Cushing's disease using cortisol, desmopressin and coupled dexamethasone-desmopressin tests: a head-to-head comparison". *Clinical Endocrinology* 83 (2015): 216-222.

28. Lonser RR., *et al*. "Outcome of surgical treatment of 200 children with Cushing's disease". *The Journal of Clinical Endocrinology and Metabolism* 98 (2013): 892-901.

29. Losa M., *et al*. "Persistent adrenocorticotropin response to desmopressin in the early postoperative period predicts recurrence of Cushing's disease". *Journal of Clinical Endocrinology and Metabolism* 94 (2009): 3322-3328.

30. Losa M., *et al*. "Desmopressin stimulation test before and after pituitary surgery in patients with Cushing's disease". *Clinical Endocrinology* 55 (2001): 61-68.

31. Magiakou MA and Chrousos GP. "Cushing's syndrome in children and adolescents: current diagnostic and therapeutic strategies". *Journal of Endocrinological Investigation* 25.2 (2002): 181-194.

32. Mampalam TJ., *et al*. "Transsphenoidal microsurgery for Cushing disease. A report of 216 cases". *Annals of Internal Medicine* 109 (1988): 487-493.

33. Newell-Price J., *et al*. "Cushing's syndrome". *Lancet* 367 (2006): 1605-1617.

34. Nieman LK., *et al*. "Treatment of cushing's syndrome: an endocrine society clinical practice guideline". *Journal of Clinical Endocrinology and Metabolism* 100.8 (2015): 2807-2831.

35. Nieman LK., *et al*. "The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline". *Journal of Clinical Endocrinology and Metabolism* 93.5 (2008): 1526-1540.

36. Pereira AM., *et al*. "Long-term predictive value of postsurgical cortisol concentrations for cure and risk of recurrence in Cushing's disease". *The Journal of Clinical Endocrinology and Metabolism* 88 (2003): 5858-5864.

37. Pivonello R., *et al*. "The Treatment of Cushing's Disease". *Endocrine Review* 36.4 (2015): 385-486.

38. Romanholi DJ., *et al*. "Role for postoperative cortisol response to desmopressin in predicting the risk for recurrent Cushing's disease". *Clinical Endocrinology* 69 (2008): 117-122.

39. Savage MO and Storr HL. "Pediatric Cushing's disease: management issues". *Indian Journal of Endocrinology and Metabolism* 16.2 (2012): S171-S175.

40. Sonino N., *et al*. "Risk factors and long-term outcome in pituitary-dependent Cushing's disease". *The Journal of Clinical Endocrinology and Metabolism* 81 (1996): 2647-2652.

41. Stratakis CA. "Cushing's syndrome in pediatrics". *Endocrinology and Metabolism Clinics of North America* 41.4 (2012): 793-803.

42. Troshina EA and Kirilyuk ML. "Diagnostics, differential diagnosis and treatment of endogenous hypercorticism". *Problems of Endocrinology* 56.2 (2010): 53-63.

43. Valero R., *et al*. "The desmopressin test as a predictive factor of outcome after pituitary surgery for Cushing's disease". *European Journal of Endocrinology* 151 (2004): 727-733.

44. Vassiliadi DA., *et al*. "The desmopressin test predicts better than basal cortisol the long-term surgical outcome of Cushing's disease". *Journal of Clinical Endocrinology and Metabolism* 101 (2016): 4878-4885.

Volume 5 Issue 6 June 2020