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Steroidogenic Enzyme 17,20-Lyase Activity in Cortisolsecreting and Non-Functioning Adrenocortical Adenomas

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1. Introduction

The frequency of adrenal incidentalomas has been reported to range between 0.7% and 4% in abdominal computed tomography [1-5], or from 1.4% to 8.7% in autopsy series [6-8]. The widespread application of high resolution imaging techniques, such as computed tomography, magnetic resonance imaging and ultrasonography, has led to an increasing frequency of discovering adrenal incidentalomas. The majority of these lesions are of cortical origin, include non-functioning adenomas, adenomas associated with pre-clinical Cushing's syndrome, pheochromocytomas, adrenocortical carcinomas, myelolipomas, ganglioneuromas, metastatic tumors and cysts [9-13]. Adrenal incidentalomas are usually asymptomatic, however many of these lesions may secrete weak precursor hormones or active hormones in insufficient amounts to cause clinically apparent disease. There are reports of several cases of adrenal incidentaloma who had no clinical evidence of Cushing's syndrome and normal basal steroid hormone secretion, but non-suppressible serum cortisol after dexamethasone administration. These cases were regarded as "pre-clinical Cushing's syndrome" [14-16] and many of them treated by adrenalectomy, which restored a normal cortisol suppression to dexamethasone.

To study the differences between non-functioning adenomas and those associated with preclinical Cushing's syndrome, we performed a comprehensive analysis of serum steroid hormone profiles in patients with such adrenocortical tumors using a combination method of high-performance liquid chromatography(HPLC) and radioimmunoassay(RIA) [17].



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2. Materials and Methods

2.1. Subjects

We studied 22 patients with incidentally discovered adrenal masses. These lesions were seen on abdominal imaging and were diagnosed clinically and/or pathologically. From our adrenal incidentaloma cases, we selected for inclusion only patients with adrenocortical adenomas. Fifteen non-functioning adenoma cases (7 males and 8 females, mean age 59.3±13.7 yr) and seven pre-clinical Cushing's syndrome cases (2 males and 5 females, mean age 54.7±12.4 yr) were studied (Table 1). As the control group, sixteen healthy adults(8 males and 8 females, mean age 51.0±7.4 yr) were used.

Non-functioning	No	Age(yr)	Sex	Side	Size(mm)	BP(mmHg)	Operation
	1	71	Μ	right	25x15	150-80	(+)
	2	83	М	right	30x20	160-84	
	3	74	М	right	30x20	146-86	(+)
	4	61	М	left	8x8	130-70	
	5	49	Μ	right	22x13	134-78	
	6	42	Μ	left	18x12	138-80	
	7	53	М	left	19x17	142-82	
	8	87	F	right	32x15	100-80	
	9	57	F	left	12x12	140-80	
	10	53	F	right	22x18	104-60	(+)
	11	55	F	right	12x9	120-76	
	12	49	F	right	26x19	124-80	(+)
	13	42	F	left	21x18	144-84	
	14	56	F	left	32x21	128-72	(+)
	15	57	F	left	25x29	146-84	
Pre-clinical	1	41	М	left	15x10	146-88	
Cushing	2	54	Μ	left	16x11	148-84	
	3	61	F	right	18x18	130-84	(+)
	4	60	F	right	17x13	160-70	(+)
	5	59	F	right	17x15	130-82	
	6	72	F	right	18x12	156-86	(+)
	7	36	F	left	22x18	124-80	(+)

Table 1. Clinical features in cases of non-functioning adenoma and pre-clinical Cushing's syndrome

2.2. Hormonal measurements

Blood samples were obtained from patients with adrenal masses and normal subjects between 8 and 9 a.m. after an overnight fast. Serum levels of 10 steroid hormones [pregnenolone (Preg), progesterone (P), deoxycorticosterone (DOC), corticosterone (B), 17hydroxypregnenolone (17-OH-Preg), 17-hydroxyprogesterone (17-OHP), 11-deoxycortisol (S), cortisol (F), dehydroepiandrosterone (DHEA), \triangle 4androstenedione (\triangle 4A)] were determined by an HPLC/RIA method as previously described [17].

2.3. Statistical analyses

We analyzed product/precursor ratios as indices of the relative activities of adrenal steroidogenic enzymes. Data are shown as mean ± SD. Variables were compared by the Mann-Whitney U test with Bonferroni correction. P values less than 0.05 were considered statistically significant.

2.4. Results

Eleven cases of non-functioning adenomas (Group I) had normal levels in DHEA and $\triangle 4A$ compared to normal controls by simultaneous analysis (Fig.1).In 4 cases of non-functioning adenomas (Group II), however, serum levels of DHEA and $\triangle 4A$ were significantly decreased compared to normal controls by simultaneous analysis (Fig.2). Other steroid hormone levels were normal in all cases of non-functioning adenomas. Therefore, we divided these patients into two groups based on the relative activities of adrenal steroidogenic enzymes by calculating product/precursor ratios. In pre-clinical Cushing's syndrome, serum levels of DHEA and $\triangle 4A$ were significantly decreased compared to normal control values (Fig.3) and these results were similar to values in Group II patients.

17,20-Lyase activities assessed by DHEA/17-OH-Preg and \triangle 4A/17-OHP ratios were significantly decreased in Group II of non-functioning adenoma and pre-clinical Cushing's syndrome compared to Group I patients and normal controls (Fig.4). 17-Hydroxylase activities assessed by 17-OH-Preg/Preg and 17-OHP/P ratios (Fig.5), 3β-hydroxysteroid dehydrogenase activities assessed by P/Preg, 17-OHP/17-OH-Preg and \triangle 4A/DHEA ratios (Fig.6), 21-hydroxylase activities assessed by DOC/P and S/17-OHP ratios (Fig.7) and 11β-hydroxylase activities assessed by B/DOC and F/S ratios (Fig.8) had the same levels in all cases of non-functioning adenomas and pre-clinical Cushing's syndrome compared to normal control values.

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Non-functioning adrenocortical adenoma (Group I)
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Cholesterol

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17 \alpha -hydroxypregnenolone
                                                                         Dehydroepiandrosterone
Pregnenolone
                     \rightarrow
                                                                  \rightarrow
0.78 \pm 0.19 ng/ml
                                      1.09 \pm 0.24 ng/ml
                                                                                   3.2\pm0.8 ng/ml
(0.74 \pm 0.14)
                                      (0.99 \pm 0.15)
                                                                                       (3.1 \pm 0.5)
                                         t
      t
                                                                                t
                          17\alpha-hydroxyprogesterone \rightarrow
                                                                   \triangle 4androstenedione
Progesterone
                  \rightarrow
0.19\pm0.10 ng/ml
                                  1.18\pm0.27 ng/ml
                                                                   1.00\pm0.28~\mathrm{ng/ml}
 (0.17 \pm 0.03)
                                        (0.94 \pm 0.20)
                                                                      (0.98 \pm 0.29)
      1
                                         l
Deoxycorticosterone
                               11-deoxycortisol
0.058±0.021 ng/ml
                                    0.93 \pm 0.27 ng/ml
 (0.060 \pm 0.013)
                                         (0.76 \pm 0.12)
       T
                                         T
Corticosterone
                                     Cortisol
1.54\pm0.38 ng/ml
                                     9.9 \pm 1.8 \ \mu \, \mathrm{g/dl}
 (1.52 \pm 0.37)
                                      (9.7 \pm 1.3)
       t
                                                                          ): Normal control
18-hydroxycorticosterone
                                                                    (
       t
                                                          *p<0.05 vs Normal control
Aldosterone
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Non-functioning adrenocortical adenoma (Group II) Cholesterol t Pregnenolone \rightarrow 17 α -hydroxypregnenolone \rightarrow Dehydroepiandrosterone $0.83 \pm 0.18 \text{ ng/ml}$ 1.10 ± 0.31 ng/ml 1.5 ± 0.8 ng/ml* (0.74 ± 0.14) (3.1 ± 0.5) (0.99 ± 0.15) T t ſ Progesterone \rightarrow 17α -hydroxyprogesterone \rightarrow \triangle 4androstenedione $0.22 \pm 0.18 \text{ ng/ml}$ $1.08 \pm 0.19 \text{ ng/ml}$ 0.32±0.13 ng/ml* (0.17 ± 0.03) (0.94 ± 0.20) (0.98 ± 0.29) l Ţ Deoxycorticosterone 11-deoxycortisol 0.072 ± 0.021 ng/ml 0.82 ± 0.18 ng/ml (0.060 ± 0.013) (0.76 ± 0.12) 1 t Corticosterone Cortisol $1.43 \pm 0.29 \text{ ng/ml}$ $9.8 \pm 2.2 \ \mu \, g/dl$ (1.52 ± 0.37) (9.7 ± 1.3) 1): Normal control (18-hydroxycorticosterone 1 *p<0.05 vs Normal control Aldosterone

Figure 2. Serum steroid hormone profiles in non-functioning adrenocortical adenoma Group II

Pre-clinical Cushing's syndrome Cholesterol l \rightarrow 17 α -hydroxypregnenolone \rightarrow Dehydroepiandrosterone Pregnenolone $0.79 \pm 0.19 \text{ ng/ml}$ 1.02 ± 0.27 ng/ml 1.7 ± 0.4 ng/ml* (0.74 ± 0.14) (0.99 ± 0.15) (3.1 ± 0.5) t t t 17α -hydroxyprogesterone $\triangle 4 and rost enedione$ Progesterone \rightarrow \rightarrow $0.30 \pm 0.14 \text{ ng/ml}^*$ $0.19 \pm 0.08 \text{ ng/ml}$ 1.12 ± 0.28 ng/ml (0.17 ± 0.03) (0.94 ± 0.20) (0.98 ± 0.29) T l 11-deoxycortisol Deoxycorticosterone 0.061±0.013 ng/ml 0.90 ± 0.27 ng/ml (0.76 ± 0.12) (0.060 ± 0.013) t t Cortisol Corticosterone 1.49 ± 0.36 ng/ml $10.1 \pm 1.9 \ \mu \, g/dl$ (1.52 ± 0.37) (9.7 ± 1.3) t): Normal control 18-hydroxycorticosterone (t *p<0.05 vs Normal control Aldosterone

Figure 3. Serum steroid hormone profiles in pre-clinical Cushing's syndrome

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Figure 4. 17,20-Lyase activities





Figure 6. 3β - Hydroxysteroid dehydrogenase activities



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Figure 8. 11β -Hydroxylase activities

3. Discussion

We examined serum steroid hormone profiles of patients with non-functioning adrenocortical adenomas and pre-clinical Cushing's syndrome. We also assessed the steroidogenic enzymatic activities of these patients by analysis of the product/precursor ratios of C21 and C19 steroid hormones in the steroidogenic pathway. Patients with pre-clinical Cushing's syndrome and a subgroup of patients with non-functioning adrenocortical adenomas had distinctly decreased adrenal androgen secretion compared with a suppressed 17,20-lyase activity. The remaining patients with non-functioning adrenocortical adenomas had completely normal adrenal androgen values and 17,20-lyase activity.

Previous reports have shown an exaggerated response of 17-hydroxyprogesterone after ACTH stimulation in some patients with incidentally detected adrenal incidentalomas [18,19]. This response has been explained by existing 21-hydroxylase deficiency which may be a pathogenetic factor in the development of adrenal tumors. Macronodular adrenal hyperplasia is a frequent finding in patients with classic congenital adrenal hyperplasia, how-ever, also about half of the heterozygous carriers in families with congenital adrenal hyperplasia were reported to have uni- or bilateral adrenal nodules on abdominal computed tomography in consecutive 4-mm scans [19]. These investigators concluded that mild 21-hydroxylase deficiency is associated with the formation of adrenal incidentaloma. Other investigators have reported that the ACTH-stimulated 17-hydroxyprogesterone levels were abnormally increased in more than 50% of patients with non-hyperfunctioning adrenal adenomas [20]. After unilateral adrenalectomy, this hormonal abnormality disappeared in most patients with adrenal tumors. The ACTH-stimulated 17-hydroxyprogesterone levels signifi-

cantly correlated with the size of the tumors. These results indicate that tumors themselves may be responsible for the increased ACTH-stimulated 17-hydroxyprogesterone levels in patients with non-hyperfunctioning adrenal adenomas.

In this study, we demonstrated that serum DHEA and $\triangle 4A$ levels were significantly decreased in some patients with non-functioning adrenocortical adenomas(Group II) and all those with pre-clinical Cushing's syndrome. 17,20-Lyase activities were significantly decreased in Group II of non-functioning adenoma and pre-clinical Cushing's syndrome compared to Group I patients and normal controls. We found no clinical or pathological differences between Group I patients and Group II patients who had normal adrenal androgen concentrations. We have no explanation as to why there was difference between these groups. Since serum steroid hormone profiles had a similar pattern between some non-functioning adrenocortical adenomas and adenomas of patients with pre-clinical Cushing's syndrome, we suggest that the former may in time progress into pre-clinical and clinical Cushing's syndrome. There are limitations in this study such as small sample size and no tissue investigation. In the future the activity of steroidogenesis enzyme should be measured in the specimens from adrenaltissues.

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