
ONCOLOGY

Serum Interleukin-6 in Patients with Adrenal Tumors

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The mean blood content of interleukin-6 in patients with adrenal tumors was much higher than in healthy donors. No correlations were revealed between interleukin-6 level, patients' sex and age, stage and duration of the disease. Interleukin-6 concentration was maximum in patients with adrenocortical cancer. A negative correlation was found between interleukin-6 level and blood cortisol concentration in patients with cortisol-producing adenoma. In patients with aldosterone-producing adenoma, interleukin-6 level tended to correlate negatively with plasma renin activity.

Key Words: *interleukin-6; adrenocortical adenoma; adrenocortical cancer; pheochromocytoma*

Growth factors and cytokines play a role in the regulation of tumor growth and dissemination. The cytokine interleukin-6 (IL-6) was first described as a factor secreted by T cells [7]. Published data show that IL-6 is mainly produced by lymphocytes, macrophages, and monocytes [12]. This cytokine is also expressed in endotheliocytes, hepatocytes, follicular cells of the thyroid gland, hypothalamic neurons, anterior pituitary cells [4-6], and histologically different tumor cells [1,6,9,11]. In human adrenals, IL-6 is primarily expressed by hormone-producing cells of the zona fasciculata and zona reticularis, but weak expression of this cytokine is noted in cells of the zona glomerulosa and catecholamine-producing cells of the medulla [8]. *In vitro* studies showed that basal and ACTH-stimulated secretion of cortisol, dehydroepiandrosterone, androstenedione, and to a lesser extent, of aldosterone increases during incubation of adrenocortical cells with IL-6 [3]. These data suggest that IL-6 is a potent auto- and paracrine regulator of steroidogenesis in the adrenal cortex. It was hypothesized that changes in the

expression of IL-6 and its receptor are a key stage in tumor transformation [2,13]. NCI-H295 cells of adrenocortical adenoma and adrenocortical cancer *in vitro* produce IL-6 [13]. We found no published data on IL-6 content in the serum from patients with adrenal tumors.

Here we measured IL-6 concentration in the serum from patients with adrenal tumors. The main clinical and morphological characteristics of this disease were taken into account.

MATERIALS AND METHODS

We examined 65 patients (44 women and 21 men, 21-75 years) with adrenal tumors hospitalized at the M. F. Vladimirkii Moscow Regional Clinical Research Institute and N. N. Blokhin Russian Oncological Center (1998-2002). The time from the onset of complaints and start of therapy varied from 2 months to 20 years. Clinical diagnosis was made for the first time and confirmed by biochemical tests, hormone assay, and histological examination of the adrenal glands after adrenalectomy. Adrenal tumors were verified by criteria of the International Histological Typing of Endocrine Tumors [10]: adrenocortical adenoma

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($n=38$), black adenoma ($n=1$), adrenocortical cancer (ACC, $n=13$), pheochromocytoma (PC, $n=11$), and myelolipoma ($n=2$).

Serum samples taken from 32 conventionally healthy donors (21 women and 11 men, 19-70 years) served as the control.

Serum IL-6 concentration was measured in fasting patients (before therapy) and healthy donors. Enzyme immunoassay was performed with R&D kits. The contents of ACTH, cortisol, and aldosterone and renin activity in the plasma and serum were measured by enzyme immunoassay with Boehringer Mannheim reagents. Fluorometry was used to estimate 24-h urinary catecholamine excretion (epinephrine and norepinephrine).

The results were analyzed by means of SAS software.

RESULTS

IL-6 concentration in conventionally healthy donors varied from 0.2 to 2.8 ng/ml (1.3 ± 0.9 ng/ml). No correlation was revealed between serum IL-6 level, sex, and age of patients.

In patients with adrenal neoplasms IL-6 concentration varied from 0.3 to 33.5 ng/ml (6.0 ± 1.6 ng/ml) and surpassed the control ($p < 0.05$). It should be emphasized that IL-6 concentration did not depend on patients' sex. IL-6 level in men and women was 0.6-33.5 ng/ml (8.2 ± 4.0 ng/ml) and 0.3-17.8 ng/ml (4.6 ± 1.1 ng/ml), respectively. We revealed no correlations between IL-6 content, sex, and duration of the disease.

IL-6 was detected in 99% patients with ACC. IL-6 concentration was highest in these patients (Table 1). The stage and functional activity of tumors determine the clinical course and prognosis of ACC. By the start of therapy the severity of ACC corresponded to stages I (T1N0M0, $n=1$), III (T3N0M0, $n=9$), and IV (T3N0M1, T4N0M1, T4N1M1; $n=3$). Serum IL-6 content did not depend on the stage of ACC. Blood IL-6 concentration in patients with ACC of stages I and III was 2.7 and 2.4-33.5 ng/ml (10.1 ± 6.3 ng/ml), respectively. Patients with ACC of stage IV had primary adrenal tumors and metastases in the liver ($n=1$) or lungs ($n=2$). In these patients IL-6 level was 3.0, 2.7, and 10.7 ng/ml, respectively. Eight patients with ACC had no clinical and laboratory markers of hormonal activity of the tumor. IL-6 concentration in these patients varied from 2.4 to 33.5 ng/ml (9.9 ± 6.0 ng/ml). In women with ACC and Cushing's syndrome (21, 52, and 58 years) serum IL-6 content was 2.7, 3.0, and 9.5 ng/ml, respectively. In one woman with ACC and Conn's syndrome (39 years) IL-6 level was 5.5 ng/ml. Blood IL-6 concentration in a woman with virile syndrome (43 years) was below the sensitivity limit of enzyme immunoassay.

IL-6 was detected in all patients with Cushing's syndrome and cortisol-producing adenoma. The mean concentration of IL-6 in these patients significantly surpassed the control values (Table 1). IL-6 concentration was highest in a woman with black adenoma (17.8 ng/ml). The content of IL-6 was minimum in a woman, who had the tumor with cortical, neuroendocrine, and neurogenic cells, pronounced polymorphism, and atypical nuclei in tumor cells (1.4 ng/ml).

Blood cortisol level in patients with cortisol-producing adenoma varied from 221 to 632 nmol/liter (422 ± 166 nmol/liter). Cortisol concentration in the blood from healthy donors was 190-750 nmol/liter. A negative correlation was found between IL-6 level and serum cortisol concentration ($r = -0.9$). Tumor size was 2.3-4.0 cm. IL-6 concentration did not correlate with tumor size. Histological examination of samples from 2 patients revealed invasion of tumor cells in the capsule of adenoma, which serves as a marker for the unknown malignancy potential [10]. Blood IL-6 content in patients (men, 33 years) was 3.5 and 8.0 ng/ml.

IL-6 was detected in 45% patients with PC. The mean content of IL-6 in these patients was higher than in the control (statistically insignificant, Table 1). IL-6 concentration was highest in a woman (40 years, 10.2 ng/ml) with extraadrenal PC.

We showed that 24-h urinary excretion of epinephrine and norepinephrine in patients with PC was 36-1146 and 71-3369 nmol, respectively. In healthy donors these indexes were 11-44 and 47-236 nmol, respectively. No correlation was found between blood IL-6 concentration and catecholamine excretion. In patients of this group, serum IL-6 level negatively correlated with serum cortisol concentration ($r = -0.6$).

Tumor size in these patients varied from 4 to 10 cm (5.6 ± 1.0 cm). Blood IL-6 level did not correlate with tumor size.

It is difficult to verify malignancy of adrenomedullary tumors. The presence of metastases in lymph nodes and distant organs is a criterion of malignancy of PC. Histologically verified invasion of surrounding tissues with tumor cells does not illustrate malignancy of the tumor [10]. We did not examine patients with pheochromoblastoma. Histological assay revealed invasion of the tumor capsule and surrounding adipose tissue with tumor cells. IL-6 was not detected in the blood from one woman (43 years). Cytokine concentration in the blood from one woman (66 years) and one man (51 years) was 6.2 and 7.6 ng/ml, respectively.

IL-6 was detected in 71% patients with aldosterone-producing adenoma of the adrenal glands. IL-6 concentration was similar in these patients (Table 1) and did not differ in men (0.6-12.5 ng/ml, 3.8 ± 3.1 ng/ml) and women (0.3-13.9 ng/ml, 4.0 ± 1.4 ng/ml).

TABLE 1. IL-6 Concentration in the Serum from Patients with Adrenal Tumors

Nosological form	Number of patients			IL-6 level, ng/ml	
	total	men	women	limits of variation	$M \pm m$
ACC	13	5	8	2.4-33.5	8.3-4.6
Cushing's syndrome	3		3	2.7; 3.0; 9.5*	
Conn's syndrome	1		1	5.5*	
virile syndrome	1		1	Not found	
hormonally inactive	8	5	3	2.4-33.5	9.9±6.0
Adrenocortical adenoma	39	10	29	0.3-17.8	5.4±2.1
Cushing's syndrome	5	2	3	1.4-17.8	6.6±5.3
Conn's syndrome	29	7	22	0.3-13.9	3.9±1.3
hormonally inactive	5	1	4	1.9; 4.7*	
PC	11	4	7	0.3-10.2	5.4±2.0
Myelolipoma	2	1	1	1.2; 1.9*	

Note. *Individual characteristics. Sign “;” separates data for different patients.

No correlations were revealed between IL-6 level, age of patients, and duration of the disease.

Blood aldosterone level in patients with aldosterone-producing adenoma was 0.09-4.20 nmol/liter (0.96 ± 0.40 nmol/liter). Aldosterone concentration in the blood from healthy donors was 0.14-1.24 nmol/liter. Plasma renin activity in patients varied from 0.4 to 3.2 ng/ml/h (1.8 ± 0.4 ng/ml/h). In healthy donors this index was 1.5-5.7 ng/ml/h. IL-6 level tended to correlate negatively with plasma renin activity ($r = -0.4$). IL-6 content did not depend on blood aldosterone concentration. IL-6 was detected only in 2 of 5 patients with hormonally inactive adrenocortical adenoma (40%, Table 1).

In patients with myelolipoma of the adrenal glands (man, 21 years; woman, 52 years) IL-6 level was 1.9 and 1.2 ng/ml, respectively.

Our results indicate that in most patients with adrenal tumors serum IL-6 content was higher than in healthy donors. No correlations were revealed between IL-6 level, patients' sex and age, and duration of the disease. IL-6 concentration was highest in patients with ACC. It should be emphasized that cytokine content did not depend on the stage of ACC. A negative correlation was found between IL-6 level and serum cortisol concentration in patients with cortisol-producing adenoma and PC. In patients with aldosterone-producing adenoma, IL-6 concentration tended to correlate negatively with plasma renin activity. The data

suggest that IL-6 is involved in the pathogenesis of adrenal tumors and plays a role in the regulation of their functional activity. This problem requires detailed investigations.

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