ONCOLOGY

Vascular Endothelium Growth Factor in the Sera of Patients with Adrenal Tumors

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 140, No. 8, pp. 198-200, August, 2005
Original article submitted May 6, 2005

Serum levels of vascular endothelium growth factor were measured in 43 patients with adrenal tumors and 25 healthy subjects. The mean blood levels of the factor in patients with adrenal tumors significantly surpassed the control. No correlations between the level of vascular endothelium growth factor, patient's age and sex were detected. The levels of this factor were maximum in patients with adrenocortical cancer, but its mean level differed negligibly from that in other morphological variants of tumors. The level of vascular endothelium growth factor tended to increase with increasing the stage of adrenocortical cancer. A direct correlation was revealed between the level of vascular endothelium growth factor and tumor size in adrenocortical cancer and aldosterone-producing adenoma. Presumably, vascular endothelium growth factor is involved into mechanisms of growth, invasion, and metastatic growth of adrenocortical cancer.

Key Words: *angiogenesis; vascular endothelium growth factor; adrenal tumors*

Recent numerous experimental and clinical findings indicate that angiogenic phenotype of the tumor evaluated by the number and density of vessels and by the level of angiogenic and antiangiogenic factors expression largely determines biological behavior of the tumor and prognosis of the disease.

Many known growth factors and cytokines are involved into regulation of angiogenesis: bFGF, aFGF, EGF, TGF-α, TGF-β, TNF, HGF, angiogenin, IL-8, thrombospondin, endostatin, IFN-γ, *etc.* [1]. However, vascular endothelium growth factor (VEGF) is considered to be the most important proangiogenic factor. The level of VEGF expression in the majority of organs (except the uterus and ovaries) is minimum. Minor expression of VEGF in human adrenals is detected in hormone-producing cortical cells [2], but in catecholamine-producing cells of the medullary layer this factor is absent [7]. The role of VEGF in the pathogenetic mechanisms of adrenal tumors is not quite clear. Angiogenesis is believed to be the main stage in the progress of tumors of the endocrine organs, obligatory for tumor cell proliferation and for hormone secretion by them [9]. Tumors of the adrenal cortex and medulla express VEGF [4,7]. However, the data on serum content of VEGF in patients with adrenal tumors are scanty and contradictory.

We compared VEGF levels in the sera of patients with adrenal tumors with consideration for clinical, hormonal, and morphological characteristics of the disease.

MATERIALS AND METHODS

Forty-three patients (37 women and 6 men aged 20-76 years) with primary tumors of the adrenals, treated at Surgical Endocrinology Department of M. F. Vladimirskii Moscow Regional Research and Clinical In-

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0007-4888/05/1402-0228

stitute in 1998-2004, were examined. The disease was clinically diagnosed for the first time in all patients; no specific treatment was carried out before the study. Functional activity of the tumor was evaluated by clinical data, biochemical findings, and hormone measurements. Topical diagnosis was made using ultrasonic examination, computer-aided tomography, magnetic resonance tomography, and angiography (if indicated). The following tumors were histologically verified after adrenalectomy: adrenocortical adenoma (n=32), adrenocortical cancer (n=8), and pheochromocytoma (n=3).

For control, VEGF levels were measured in the sera of 25 normal subjects (13 women and 12 men aged 17-67 years).

Serum concentration of VEGF before therapy and in controls was measured by enzyme immunoassay using R&D reagent kits. The concentrations of ACTH, hydrocortisone, and aldosterone and renin activity were measured in the serum and plasma using Boehringer Mannheim reagents, daily excretion of catecholamines (epinephrine, norepinephrine) was measured by the fluorimetric method.

The data were statistically processed using Statistica, Release 6.0, and STARSOFT software. The significance of differences was evaluated using Student's t test (the differences were considered significant at $p<0.05$).

RESULTS

Serum levels of VEGF in healthy subjects varied from 20.6 to 461.9 pg/ml, the mean value being 150.8±22.7 pg/ml (median 126.5 pg/ml).

The range of values in patients with adrenal tumors was greater: 15.8-1052.0 pg/ml, the mean level (289.7±34.2 pg/ml) and the median (237.6 pg/ml) being significantly higher than in the control ($p<0.008$). Analysis of the distribution of VEGF values showed that the concentration of this growth factor in the serum surpassed 200 pg/ml in 63% patients with adrenocortical cancer and in 59% patients with adrenocortical adenoma, while in the group of normal subjects this VEGF level was noted in only 34% cases ($p<0.05$). No correlation between VEGF level, age and sex were detected in patients and controls.

The highest VEGF levels were detected in patients with adrenocortical cancer, but no significant differences between the means in patients with adrenocortical cancer, adenoma, and pheochromocytoma were detected (Table 1).

Serum level of VEGF tended to increase with increasing the stage of adrenocortical cancer. In stage I (n=1) VEGF level was 180.7 pg/ml, in stage III (n=4) 392.6 pg/ml, and in stage IV (n=3) 589.9 pg/ml.

Tumor size in patients with adrenocortical cancer was 3.5-15.0 cm. A direct correlation between VEGF level and tumor size was detected (r=0.68; p=0.2).

Four patients with adrenocortical cancer had no clinical manifestations or laboratory data indicating functional activity of the tumor. Serum VEGF levels were maximum in these patients (185.7-1052.0 pg/ml), the mean level (654.8±252.6 pg/ml) and the median (726.5 pg/ml) significantly surpassed the control (p=0.046). The mean level of VEGF in hormonally inactive cancer was higher than in patients with adrenocortical cancer with Cushing's syndrome (370.9± 78.3 pg/ml, range of values 180.7±531.9 pg/ml, median 385.6 pg/ml), but the differences were statistically negligible (p=0.27) because of low number of observations. In a female patient with adrenocortical cancer and virile syndrome the level of VEGF (72.9 pg/ml) did not differ from that in the control group.

Patients with adrenocortical adenoma were divided into 3 groups, depending on functional activity of the tumor: patients with hydrocortisone-producing (n=8), aldosterone-producing (n=17), and hormonally inactive adenoma (n=7).

Serum VEGF levels in patients with hydrocortisone-producing adenoma varied from 52.3 to 674.4 pg/ml, the mean level being 268.3±71.9 pg/ml (median 244.9 pg/ml). No significant differences in the mean levels of VEGF in adrenocortical cancer concomitant with Cushing's syndrome and in hydrocortisone-producing adenoma were detected. The diameters of tumors in these patients were 2.5-7.0 cm. No correlation between VEGF content and tumor size

TABLE 1. Serum Levels of VEGF in Patients with Adrenal Tumors and Normal Subjects

Histological type of tumor	Number of patients	Age	VEGF level, pg/ml		
			$M±m$	range	median
Control (normal subjects)	25	38.7±6.2	150.8±22.7	20.6-461.9	126.5
Adrenocortical cancer	8	49.4±4.8	440.1±115.6**	72.9-1052.0	285.6
Adrenocortical adenoma	32	49.3±6.4	247.0±28.7*	15.8-674.4	226.0
Pheochromocytoma	3	46.3±3.4	343.2±220.1	97.8-782.6	149.3

Note. *p=0.042, **p=0.0039 compared to the control.

were detected. No relationship between VEGF content, blood hydrocortisone concentration, and daily excretion of this hormone was found.

The mean serum level of VEGF in patients with aldosterone-producing adenoma was 239.0±37.1 pg/ml (18.8-558.1 pg/ml, median 204.0 pg/ml). No correlation between VEGF level and aldosterone content in the blood was detected. A significant correlation between VEGF level and tumor size was detected in these patients (r=0.44; p=0.15).

The levels of VEGF in patients with hormonally inactive adenoma varied from 15.8 to 509.3 pg/ml, the mean level being 242.3±59.8 pg/ml (median 250.1 pg/ml). Tumor diameters in these patients were 2.0-7.0 cm. No correlation between serum VEGF level and tumor size was detected.

The level of VEGF in patients with pheochromocytoma varied within a wide range and did not differ significantly from this parameter in controls. Histological study revealed signs of invasive tumor growth in 2 cases. In one female patient (41 years) pheochromocytoma (8 cm in diameter) of solid structure was characterized by invasion of tumor cells into the tumor capsule and angioinvasion. Serum VEGF content in this patient (97.8 pg/ml) did not differ from that in women from the control group. In another case pheochromocytoma (tumor diameter 6.5 cm) with alveolar structure was verified with tumor cell invasion in the tumor capsule and adjacent fatty tissue. Serum VEGF level in this female patient (59 years) was 782.6 pg/ml.

Hence, the mean content of VEGF in the sera of patients with adrenal tumors was significantly higher than in normal subjects. The highest levels of VEGF were detected in patients with adrenocortical cancer, but no significant differences in the mean levels of this factor in patients with adrenocortical cancer, adenoma, and pheochromocytoma were detected.

The increase in the level of circulating VEGF was observed in patients with tumors of different histogenesis and location; according to published data, its serum level can be regarded as a criterion characterizing tumor malignancy and disease prognosis [3,5, 6,8]. Because of little number of observations we cannot evaluate properly clinical and prognostic significance of this growth factor in adrenal tumors. However, the detected trend to increase in serum VEGF level in patients with higher stages of adrenocortical cancer and direct correlation between its content and tumor size suggest that VEGF is involved into mechanisms of adrenocortical cancer growth, invasion, and metastatic growth.

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