Serum sFas, Leptin, and VEGF in Patients with Ovarian Cancer and Benign Tumors

M. M. Vysotskii, M. A. Digaeva, N. E. Kushlinskii, S. G. Abbasova, K. P. Laktionov*, V. D. Ermilova*, K. A. Bakhoeva, Yu. V. Kryuk, and I. B. Manukhin**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 11, pp. 564-568, November, 2009
Original article submitted July 28, 2009

The initial levels of soluble Fas antigen (sFas), leptin, and vascular endothelium growth factor (VEGF) were measured in the sera of 100 patients with ovarian cancer and benign tumors and in 60 healthy women aged 28-65 years. Serum levels of sFas and VEGF were elevated in the total group of patients with ovarian tumors, while leptin levels were the same as in healthy women. The studied parameters did not depend on the age of patients and healthy women. The levels of sFas and leptin were virtually the same in benign and malignant ovarian tumors, while VEGF concentration was higher in patients with ovarian cancer. The mean serum levels of sFas, VEGF, and leptin in patients with poorly and moderately differentiated serous ovarian cancer were 2-fold higher than in well-differentiated tumors ($p<0.05$), while serum concentrations of sFas and leptin increased with the disease stage progress in patients with ovarian cancer ($p<0.05$). According to the data of unifactorial analysis, the increase in serum levels of sFas and VEGF in ovarian cancer patients correlated with short duration of the relapse-free period. Multifactorial analysis showed that the disease stage ($p=0.006$), presence of ascites ($p=0.03$), VEGF concentration ($p=0.02$), and the sFas/leptin coefficient ($p=0.045$) are highly significant independent factors for predicting the relapse-free survival of patients with serous ovarian cancer.

Key Words: *ovarian cancer and benign tumors; sFas; VEGF; leptin; prognosis*

Ovarian cancer ranks first in the structure of mortality from malignant tumors of the female reproductive system [2]. Analysis of the Russian and foreign publications showed unsatisfactory results of treatment of patients with ovarian cancer, because by the moment of diagnosis about 70% patients have advanced stages of the tumor process [3]. In addition, ovarian cancer unites the histogenetic variants of tumors, the majority of which are characterized by aggressive clinical course and are liable to early metastasizing. The aggressive nature of the tumor manifests by its capacity to invasion and metastasis development. This can be due to histogenesis, differentiation degree, and genome damage, all this leading to activation of the factors closely related to "biological" behavior of the tumor.

Studies of biological factors essential for tumor growth and metastasizing will lead to better understanding of some stages in the pathogenesis of ovarian tumors. Inhibition of Fas-dependent apoptosis is an important component in the system of transformed cell defense from antitumor immunity. The Fas receptor triggers apoptosis in the target cell after interactions with its ligand (FasL). Soluble Fas (sFas) distantly

Moscow State Medical Stomatological University; *N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow; **Affiliated Department of M. M. Shemyakin and Yu. A. Ovchinnikov Institute of Organic Biochemistry, Russian Academy of Sciences, Pushchino, Russia. ***Address for correspondence:*** biochimia@mtu-net.ru. N. E. Kushlinskii

0007-4888/09/1485-0810 © 2009 Springer Science+Business Media, Inc.

inhibits FasL-induced apoptosis by providing advantages in survival and multiplication for sFas producer cells [5]. High serum level of sFas is associated with unfavorable prognosis of prostatic cancer [6], non-Hodgkin lymphoma [7], mature T-cell leukemia [8], urinary bladder cancer [11], and melanoma [10].

Leptin (a hormone of the white adipose tissue promoting reduction of fat reserve, including that at the expense of adipocyte apoptosis [9]) plays an important role in the regulation of human need in nutrients and energy expenditure. Leptin is secreted by the white fatty tissue and bone marrow adipocytes, placental trophoblasts and uterine amnionic cells in pregnant women, is involved in the regulation of steroidogenesis [4]. The majority of leptin studies were carried out on cell cultures, while clinical studies were just solitary, for example, studies of its relationships with apoptosis stimulants and inhibitors in patients with ovarian cancer.

Studies of neoangiogenesis mechanisms in the pathogenesis of tumors attract great attention of scientists. Vascular endothelial growth factor (VEGF) is assumed to be the most active stimulant of tumor angiogenesis [12].

We measured serum levels of sFas, leptin, and VEGF in patients with ovarian cancer and evaluated their role in the clinical course and prognosis of this disease.

MATERIALS AND METHODS

Serum concentration of sFas in patients and healthy women were measured as described previously [1]. Monoclonal antibodies (MAb) to Fas SA-8 (IgG1 (κ); $K_a=(8.8\pm0.6)\times10^7$) were adsorbed on EIA plates (Linbro) in 0.05 M carbonate buffer (pH 9.6), 1 μg/well, overnight at 4°C. Free binding centers on the plates were blocked with 1% BSA in PBS (pH 7.2) for 1 h at 37°C. The sera from patients and healthy women (control) were then pipetted into the plates. Full-length recombinant Fas in serial 2-fold dilutions (40-0.07 ng/ml) was pipetted into each plate for positive control. The plates were incubated for 1.5 h at 37°C and then intensely washed several times in PBS with 0.1% Twin-20 (PBST; Sigma). The washing procedure was repeated after each stage of the test. After washing biotin-conjugated MAb to Fas SA-7 (IgG1 (κ); $K_a=(9.52\pm1.4)\times10^8$) in a concentration of 10 μg/ml in PBST solution with 0.1% BSA were added. The plates with biotin-conjugated antibodies were incubated for 2 h at 37°C. Streptavidin peroxidase solution (Amersham) in the recommended dilution in PBST was then added into the plates and incubated for 1 h at 37°C. Fresh 0.04% orthophenylene diamine solution in 50 mM citrate phosphate buffer (pH 5.0) with 0.03% hydrogen peroxide served as the substrate. The plates were incubated for 15-20 min at ambient temperature for staining development. The reaction was stopped by adding sulfuric acid. Optical density was measured at λ=492 nm on an MR 700 Microplate Reader (Dynatech Labs). Serum concentrations of sFas were evaluated by calibration curves corresponding to each plate. Serum leptin concentrations were measured by enzyme immunoassay using DRG kits, VEGF using CytElisa™ Human VEGF reagents (Cytimmune Science Inc.).

The means and errors of the means were calculated for the parameters; the median was calculated for log-normal distribution. The differences were considered significant at $p<0.05$. For comparison of more than 2 groups, the p values were calculated with amendments for multiple comparisons. Correlations were evaluated using parametric Pearson test. Partial correlation coefficient was calculated for related parameters. In order to detect the most common relationships between the totality of signs, "factorial analysis" was carried out by singling out the main components with subsequent rotation of the correlation matrix. The factors with the greatest relationship with the studied parameters were detected by multifactorial analysis of dispersions. Statistical analysis was carried out using Statistica software.

RESULTS

Serum concentrations of sFas, leptin, and VEGF were measured before therapy in 100 women with ovarian tumors, examined and treated at Institute of Clinical Oncology, N. N. Blokhin Cancer Research Center, Hospital of War Veterans No. 2, Department of Public Health of Moscow, and Center of Obstetrics, Gynecology, and Perinatology from January 2000 to December 2005. Clinical diagnosis of ovarian tumors was confirmed by morphological findings in accordance with histological "Classification of Malignant Tumors" (WHO, 1999).

The total group included patients with serous cancer (n=51), borderline tumors (n=11), and benign ovarian tumors (n=38). The mean age of the patients varied from 28 to 65 years. The groups of patients were similar by age. Sixty women with ovarian tumors were in a postmenopause of 1.5 to 15 years.

Patients with FIGO stages III (32 women) and IV (14 women) and with poorly differentiated tumors (38 women) predominated in the group of patients with serous ovarian cancer; 2 patients presented with stage I and 3 with stage II. The tumors were poorly differentiated in the majority of women (n=38); moderately differentiated tumors were detected in 7, well-differentiated in 5 patients. The differentiation degree

TABLE 1. Serum sFas Levels in Patients with Ovarian Tumors and Healthy Women (*M*±*m*)

Group	Incidence of sFas, %	sFas, ng/ml	Range
Healthy women (control), *n*=60	36.0	0.86±0.3	0.3-1.2
Total group of patients, *n*=100	77.0	2.31±0.61	0.4-5.6
Serous ovarian cancer, *n*=51	72.9	2.09±0.45	0.4-5.6
Borderline tumors, *n*=11	75.0	3.58±1.35	0.87-4.94
Benign ovarian tumors, *n*=38	78.6	2.21±0.43	0.9-4.9

could not be evaluated in 2 cases. The overwhelming majority (89%) of patients had ascites.

Radical interventions were carried out in all patients with borderline and benign ovarian tumors. Cytoreductive surgical interventions were carried out in patients with ovarian cancer with consideration for the tumor process stage.

After surgery all patients with ovarian cancer monthly received courses of polychemotherapy including platinum preparations and taxane derivatives according to common protocols. The patients received 6-12 courses of adjuvant polychemotherapy.

Serum concentrations of sFas (Table 1), leptin, and VEGF were measured in all patients with ovarian tumors at first examination. No appreciable differences in sFas concentrations in different groups of patients were detected, but the levels of sFas in all groups of patients differed significantly from those in the control (p=0.003).

Leptin was detected in patients with ovarian tumors and healthy women. No appreciable differences in its serum levels in the groups of patients and in patients *vs.* controls were detected (Table 2). It is noteworthy that the groups of patients and healthy women virtually did not differ by Bray's index.

The sFas/leptin coefficient was calculated for patients with ovarian tumors, in whom sFas was detected. Highly significant differences in this coefficient in the total group of patients with ovarian tumors and control group were detected. No appreciable differences between its values in the main groups of patients were detected.

VEGF was detected in all patients with ovarian tumors and in healthy women. Its values varied within a great range in the total group of patients (from 80 to 860 pg/ml), its mean level being significantly higher than in the control (p<0.05; Table 2). Serum level of VEGF in ovarian cancer was significantly higher than in benign tumors and did not differ from that in patients with borderline tumors. The mean VEGF level was significantly higher in patients with serous cancer with ascites (640±50 pg/ml) than without it (410±49 pg/ml), while serum sFas and leptin levels did not depend on the presence of ascites.

No correlations between the initial concentrations of sFas, leptin, and VEGF were detected in the total group of patients. Multifactorial analysis of dispersions revealed no significant differences in the studied parameters in patients with serous cancer and benign ovarian tumors in patients of different age and with different duration of the posmenopausal period. Serum levels of sFas, leptin, and VEGF in patients with ovarian cancer increased with the disease progress (at advanced stages; Table 3) and were higher in poorly differentiated *vs*. moderately and well-differentiated tumors (p<0.05; Table 3).

Simultaneous relationships between several clinical morphological signs of the disease and levels of

TABLE 2. Serum Leptin and VEGF Levels in Patients with Ovarian Tumors and Healthy Women (*M*±*m*)

Group	Leptin, ng/ml	VEGF, pg/ml
Healthy women (control), *n*=60	6.40±0.98 (0.64-11.1)	170±14 (60-280)
Total group of patients, *n*=100	7.7±2.6 (0.63-29.5)	321±36* (80-860)
Serous ovarian cancer, *n*=51	6.9±1.7 (0.63-25.6)	630±32 (400-860)
Borderline tumors, *n*=11	8.4±3.7 (1.35-18.2)	440±65 (330-560)
Benign ovarian tumors, *n*=38	8.7±2.4 (0.86-29.5)	200±19^{+} (80-320)

Note. The range of values is shown in parentheses. *p=0.02 *vs*. control, ^{+}p=0.03 *vs*. the value in patients with serous ovarian cancer.

TABLE 3. Serum Levels of sFas, Leptin, and VEGF in Patients with Serous Ovarian Cancer with Consideration for the Main Clinical Morphological Characteristics of the Tumor (*M*±*m*)

Parameter		Number of patients	sFas, ng/ml	Leptin, ng/ml	sFas/ leptin	VEGF, pg/ml
Disease stage (FIGO)	I	2	1.17±0.4	3.03±1.4	0.39±0.1	510±107
	II	3	1.75±0.4	3.60±1.8	0.35±0.2	580±137
	III	32	2.03±0.5	7.95±2.5	0.57±0.3	650±32
	IV	14	2.36±0.9	5.69±2.1	0.90±0.5	640±37
Tumor differentiation	poor	38	2.53±0.59	7.95±2.16	0.65±0.27	680±80
	high and moderate	11	1.11±0.18	3.83±1.06	0.76±0.53	450±43
Maximum size of primary tumor						
Serous ovarian cancer	<10 cm	29	1.57±0.47	6.21±2.2	0.48±0.24	650±58
	≥10 cm	22	3.28±0.78*	7.21±2.13	1.14±0.54	680±75
Benign and borderline ovarian tumors	<10 cm	33	1.71±0.19	8.82±3.33	0.54±0.19	230±34
	≥10 cm	16	3.54±1.3^{+}	8.49±3.86	1.23±1.02	210±48
Duration of relapse-free period in patients with ovarian cancer	<12 months	36	2.53±0.87	3.80±1.66	1.35±0.50	
	≥12 months	15	1.84±0.59	9.17±2.49	0.23±0.05^{o}	

Note. *p=0.029 and ^{+}p=0.05 compared to the corresponding parameter in patients with tumors <10 cm in size, ^{o}p=0.023 compared to the corresponding parameter in patients with remission <12 months long.

sFas and leptin and their proportion in patients with serous cancer of the ovaries were evaluated. Multifactorial analysis of dispersions failed to detect combined effects of such factors as differentiation degree and size of the tumor or disease stage on significant changes in the concentrations of the studied parameters. However, the sFas/leptin coefficient significantly correlated with the disease stage (Table 3).

Relapse-free survival was analyzed in 51 patients with serous ovarian cancer. The age, tumor differentiation degree, and the presence of ascites were inessential for this parameter. The disease stage significantly (p=0.02) correlated with relapse-free survival of patients with serous ovarian cancer. Relapse-free survival of patients with stage III serous cancer was 1.10±0.05 years *vs.* 0.31±0.11 years in stage IV.

The levels of sFas, leptin, and their coefficient (proportion) were analyzed with consideration for the length of relapse-free period in patients with serous ovarian cancer. Serum level of sFas in patients with a short (<12 months) relapse free period was 1.4 times higher, of leptin 2.4 times lower, and their coefficient 5.9 times higher (p=0.023) than in patients with longer relapse-free period (more than 12 months). Patients with relapse-free period >12 months predominated (67%) among patients in whom sFas was not detected.

The initial leptin level did not correlate with relapse-free survival values in patients with serous ovarian cancer. However, the sFas/leptin coefficient <0.6 and >0.6 significantly (p=0.02) correlated with the disease prognosis, the median of relapse-free survival being 11.1±0.06 and 0.31±0.10 years, respectively.

Unifactorial analysis showed a trend to longer relapse-free survival of patients with serous ovarian cancer with low serum levels of VEGF. The parameter in patients with low VEGF levels (≤500 pg/ml) during the first 12 months from the beginning of therapy was 88.9±13.9% *vs.* 62.5±14.7% in patients with higher VEGF levels (>500 pg/ml).

Multifactorial analysis revealed a common relationship of the disease stage, length of relapse-free period, and serum VEGF in patients with ovarian cancer (p=0.004). Mainly patients with a significantly lower level of serum VEGF had favorable prognosis. The sFas/leptin coefficient in patients with serous ovarian cancer correlated only with the duration of relapse-free period (p=0.026; multifactorial analysis data). Multifactorial analysis also showed that the disease stage (p=0.006), presence of ascites (p=0.03), VEGF initial concentration (p=0.02) and sFas/leptin coefficient (p=0.045) are highly significant independent factors for prediction of relapse-free survival of patients with serous ovarian cancer.

Hence, the relationship of serum sFas, leptin, and VEGF levels in patients with serous ovarian cancer and the main clinical morphological characteristics

of the disease and the relapse-free survival prognosis were studied. The levels of sFas, leptin, sFas/leptin, and VEGF can be used as additional prognostic factors in patients with serous ovarian cancer.

REFERENCES

1. S. G. Abbasova, N. E. Kushlinskii, V. M. Lipkin, and N. N. Trapeznikov, *Byull. Eksp. Biol. Med.*, **127**, No. 3, 128-131 (1999).
2. E. M. Aksel' and M. I. Davydov, *Medical Information Agency* [in Russian], Moscow (2003), pp. 100-102.
3. K. I. Zhordania, *Rus. Med. Zh. (Oncol.)*, **10**, No. 24, 1095-1102 (2002).
4. I. V. Tereshchenko, *Probl. Endokrinol.*, **47**, No. 4, 40-46 (2001).
5. J. Cheng, T. Zhou, C. Liu, *et al.*, *Science*, **263**, 1759-1762 (1994).
6. Y. Furuya, O. Nagakawa, and H. Fuse, *Endocr. J.*, **50**, No. 5, 629-633 (2003).
7. T. Hara, H. Tsurumi, M. Takemura, *et al.*, *Am. J. Hematol.*, **64**, No. 4, 257-261 (2000).
8. S. Kamihira, Y. Yamada, M. Tomonaga, *et al.*, *Br. J. Haematol.*, **107**, No. 4, 851-860 (1999).
9. H. Masuzaki, Y. Ogawa, N. Sagawa, *et al.*, *Nat. Med.*, **3**, No. 9, 1029-1033 (1997).
10. Y. Mizutani, O. Yoshida, and B. Bonavida, *J. Urol.*, **160**, No. 2, 571-576 (1998).
11. R. Mouawad, D. Khayat, and C. Soubrane, *Melanoma Res.*, **10**, No. 5, 461-467 (2000).
12. M. Toi, S. Hoshina, T. Takayanagi, and T. Tominaga, *Jpn. J. Cancer Res.*, **85**, No. 10, 1045-1049 (1994).