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Insulin-Like Growth Factors (IGF) and IGF-Binding Proteins (IGFBP) in the Serum of Patients with Ovarian Tumors

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IGF-1, IGF-2, and IGFBP-1,2,3 were assayed in blood serum of patients with malignant ovarian tumors (n=44), borderline ovarian tumors (n=11), and benign ovarian tumors (n=12) as well as in healthy women (n=33). In blood serum of patients with malignant ovarian tumors, the level of IGF-1 was lower and IGFBP-1 was higher than in other groups. In patients with malignant and borderline ovarian tumors, the level of IGFBP-2 was higher than in healthy women and in patients with benign ovarian tumors. There was no correlation between most examined parameters and the clinical and morphological peculiarities of ovarian tumors. The study revealed IGF/IGFBP imbalance in patients with malignant ovarian tumor and showed that IGFBP-2 proved to be a potential diagnostic serological marker w with 90% sensitivity and 90% specificity.

Key Words: *insulin-like growth factors IGF-1 and IGF-2; IGF-binding proteins IGFBP-1, 2, 3; ovarian tumors*

Ovarian cancer is one of the most malignant forms of ovarian tumors (OT) of the female reproductive system, which in majority of patients is diagnosed at late stages characterized by extensive tumor dissemination over the peritoneum. Pronounced metastatic and invasive potentials of malignant OT necessitate comprehensive study of the growth and expansion mechanisms of these tumors to substantiate prognostication of the results of standard therapy and treatment with drugs affecting the regulator molecules.

The key role in the onset and progress of various malignant tumors is given to the signal system of insulin-like growth factors (IGF). It comprises IGF-1 and IGF-2, mitogen peptides that are highly homologous to insulin and to each other. They are produced in the liver and some other organs under the effect of somatotropin (growth hormone) and spread in the organism with blood, which is referred to as central or endocrine mode of action. In addition, IGF signal system includes transmembrane IGF receptors of cells and IGF-binding proteins (IGFBP) in the blood. The cells of various tumors also synthesize IGF that play the role of auto- and paracrine transmitters mediating the growth, metastasizing, and anti-apoptotic reactions of malignant cells. IGF, IGF receptors, and IGFBP constitute an intricate network of interacting with each other and other biological regulators of cell growth and survivability. At present, 6 members of IGFBP family are known, which bind IGF with equal or even greater affinity in comparison with nominal IGF receptors. IGFBP modulate the bioavailability and activity of IGF in several ways by *1*) transferring IGF from peripheral blood to the tissue targets (IGFBP-1,2,4); *2*) maintaining the reserve supply of IGF in the blood (predominantly, IGFBP-3); *3*) up- and down-regulating the IGF effects; and *4*) mediating some IGF-independent biological effects. In various physiological environments, IGFBP can either stimulate or inhibit IGF effects thereby increasing the half-life of growth factors or competing with them for common receptors. Activity of IGFBP and related cellular effects of IGF are controlled by specific proteases (in particular, by serine proteases and MMP), which augment bioavailability of IGF by hydrolyzing IGFBP to small fragments with diminished affinity to IGF.

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The IGF signal system plays important roles in normal performance of the ovaries [7] as well as in the onset and progress of malignant epithelial tumors [5]. All components of this system are expressed in OT cells where they can be viewed as significant factors in prognosis of the disease [8,11,12]. In contrast, the role of IGF and IGFBP, which circulate in the peripheral blood, in the onset and progress of OT is ambiguous. One of the most important reasons to investigate the role of IGF signal system in patients with ovarian cancer is promising possibility to employ the specific (targeted) inhibitors to suppress activity of this system [4,6,15].

Our work was designed to compare the levels of IGF-1, IGF-2, and IGFBP-1,2,3 in blood serum of the patients with OT and to analyze the interrelations of examined parameters with basic clinical and morphological peculiarities of ovarian tumor.

MATERIALS AND METHODS

The study included the patients with malignant OT (n=44), borderline OT (n=11), and benign OT (n=12) as well as the control virtually healthy women (n=33). All examine groups were age-matched. By histological structure, the benign OT belonged to serous (n=3), endometrial (n=4), and other (n=5) types. Borderline OT belonged to serous (n=7) and mucinous (n=4) types. In 72% cases, malignant OT were the serous adenocarcinomas. According to FIGO Ovarian Cancer Staging, the ovarian cancer patients had OT at stage I (n=8) or stage II (n=4). In 32 patients, ovarian cancer was extrapelvic with predominance of IIIc stage in 18 patients.

The levels of IGF-1, IGF-2, and IGFBP-1,2,3 were assayed in blood serum prior to specific treatment. To this end, we used a BEP 2000 Advance System (Siemens Healthcare) and the standard Mediagnost kits for direct ELISA according to the protocol guide.

The data were analyzed statistically using Statistica 7.0 software. Since distributions of most parameters deviated from normalcy, we employed non-parametrical Mann–Whitney and Kruskal–Wallis tests as well as Spearman's rank correlation test at $p<0.05$.

RESULTS

We observed a significant drop in median level of IGF-1 in blood serum of patients with malignant OT accompanied by an increase of IGFBP-1 level in comparison with all other groups, while the level of IGF-2 was virtually the same in all groups (Table 1). In blood serum of patients with malignant and borderline OT, the level of IGFBP-2 was higher than in the control or benign OT groups. The highest level of IGFBP-3 was observed in blood serum of healthy women, while the lowest IGFBP-3 concentration was found in patients with malignant OT, although there was no significant difference between these groups (p=0.059). Similar changes in IGF-1 level were characteristic of other tumors in the reproduction system such as breast tumor [2] and cervical carcinoma [1]. The regularities found here agree with the reported data on the serum level of IGF-1 and IGFBP in patients with malignant OT [3,9,14]. However, these data and our findings are in somewhat controversy with the results of epidemiological studies attesting to positive correlation between serum IGF-1 and the risk of malignant OT in certain age groups [10]. Still, it should be noted that analysis of 3 cohort studies did not corroborate the

TABLE 1. Serum Levels of IGF-1, IGF-2, and IGFBP-1,2,3 (ng/ml) in Patients with Benign, Borderline, and Malignant OT (Me; 25-75%)

Group	IGF-1	IGF-2	IGFBP-1	IGFBP-2	IGFBP-3
Control (N=33)	120 (104-160)	855 (618-1158)	4.21 (2.09-10.50)	144 (123-224)	4788 (4160-5550)
Benign OT (N=12)	130 (82.9-184.0)	772 (687-874)	2.49 (0.54-5.52)	202 (170-222)	1367 (1185-1855)
Borderline OT (N=11)	124 (82.7-162.0)	883 (633-1171)	5.52 (0.52-11.00)	929 (268-1121) *p=0.0009 ^{+}p=0.012	1567 (1110-2341)
Malignant OT (N=44)	86.38 (56.5-120.0) *p=0.0016 ^{+}p=0.021 ^{o}p=0.03	770 (638-1199)	16.7 (6.8-36.9) *p=0.04 ^{+}p=0.0003 ^{o}p=0.011	913 (564-1861) *p=0.00001 ^{+}p=0.001	1027 (0-1935)

Note. Significance is given in comparison with *control, $^{+}$benign, and ^{o}borderline OT.

TABLE 2. Sensitivity of IGFBP-1 and IGFBP-2 as Serological Markers of Malignant OT at Various Specificity Level

Marker	Specificity, %	Threshold, ng/ml	Sensitivity, %
IGFBP-1	95	14.9	51
	90	12.5	54
	80	12.0	56
	70	9.0	61
IGFBP-2	95	535	76
	90	320	90
	80	248	95
	70	206	95

effect of the components of IGF system on the risk of OT development [13].

In patients with benign and borderline OT, a negative correlation was revealed between serum IGF-1 on the one hand, and IGFBP-1 (r=-0.45; p=0.0004) and IGFBP-2 (r=-0.43; p=0.0008), on the other. The positive correlations were observed between the serum levels of IGF-1 and IGFBP-3 (r=0.57; p=0.00004) as well as between the serum levels of IGFBP-1 and IGFBP-2 (r=0.65; p=0.00002). Similar correlations were observed in patients with malignant OT. In contrast, the healthy women demonstrated no correlation between IGF-1 and any IGFBP, although the level of IGF-2 positively correlated with IGFBP-3. Overall, these data attest to disturbance of the balance between IGF and IGFBP in patients with all three types of OT. Indirectly, they reflect various roles played by IGFBP-1 and IGFBP-2, on the one hand, and IGFBP-3, on the other hand, in the control over IGF bioavailability.

The potentially important serological markers of ovarian cancer turned to be only IGFBP-1 and IGFBP-2, whose levels were enhanced in patients with malignant OT (both markers) and with borderline OT (IGFBP-2). To assess diagnostic potential of these markers, we calculated their sensitivity at various levels of specificity (Table 2). These data show that sensitivity of IGFBP-1 at rather sufficient level of specificity (>70%) is no more than 61%, while IGFBP-2 demonstrated a high sensitivity of 76-95% with specificity of 95-70%, respectively. Thus, the optimal threshold level of this marker is 320 ng/ml, which corresponds to 90% sensitivity and 90% specificity.

Additional evidences in favor of possibility to use IGFBP-2 as a serological marker of ovarian cancer are the positive correlation of serum IGFBP-2 with the stage of OT (r=0.52; p=0.008) and with routine ovarian cancer marker CA-125 (r=0.39; p=0.041). The levels of other IGF elements were related neither to FIGO stage nor to serum concentration of CA-125. Moreover, the detailed study of the relationships between the levels of all examined proteins on the one hand, and the basic parameters of tumor spread such as *1*) the size of primary tumor, 2) availability and character of peritoneal dissemination and metastasis in greater omentum, and *3*) occurrence and volume of ascites, on the other hand, revealed no significant correlations. In addition, there was no dependence of serum IGF/IGFBP levels on histological structure or degree of tumor differentiation.

Thus, the present comparative ELISA for serum IGF-1, IGF-2, and IGFBP-1,2,3 in patients with OT and healthy women revealed significant disturbance of IGF/IGFBP balance in patients with ovarian cancer attesting to enhancement of IGF bioavailability for tumor cells when there were no substantial changes in the level of IGF-2 or even during a drop of IGF-1 concentration. IGFBP-2 is viewed as a potential serological marker of ovarian tumor, whose level depends on the degree of the disease and correlates with that of CA-125. At 90% specificity, the sensitivity of IGFBP-2 is as high as 90%.

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