ONCOLOGY

Granulosa Cell Tumors of the Ovary and Inhibin B

N. V. Lyubimova, A. M. Beyshembaev, D. N. Kushlinskiy*, K. I. Zordania*, L. V. Adamyan*

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We present the results of immunoenzyme detection of inhibin B in blood serum of patients with adult-type granulosa cell tumors of the ovary. Blood concentration of inhibin B at the end of menstrual cycle in patients with tumor relapse was significantly higher than in patients during remission and in virtually healthy women. The increase in inhibin B concentration preceded clinical manifestation of the disease relapse by 2-13 months, which demonstrates high diagnostic sensitivity of this marker and suggests that it can be recommended for the use in diagnostics and monitoring of granulosa cell tumors of the ovaries.

Key Words: *inhibin B; granulosa cell tumors of the ovary; relapses*

Malignant tumors of the reproductive system are the most common tumors in women. According to International Agency for Research on Cancer, more than 165,000 new cases of ovary neoplasms are annually registered worldwide. In Russia, ovary tumors are detected in more than 12,300 women (16,500 per 100,000) every year; they rank 7 in total oncological morbidity (7%) and 3 in gynecological neoplasms (after endometrial cancer and cervical cancer) [1].

According to different authors, granulosa cell tumors of the ovary (GCTO) constitute 1.5 to 4.0% of all tumors of this localization and are the most common hormone-producing tumors of the ovary (~85%). According to International Histological Classification of Tumors, World Health Organization (1995), GCTO are assigned to sex cord-stromal tumors, to the group of granulosa-stromal-cell tumors of the ovary. They significantly prevail over other morphological variants of ovarian granulosa-stromal-cell tumors (thecomas, fibromas, androblastomas, and gynandroblastomas), comprising 50-55% of all these tumors [2].

GCTO are assigned to the group of unpredictable tumors, *i.e.* assessment of their malignant potential on the basis of available clinical, morphological, and biological prognosis factors is still complicated. Relapses appear in 40% patients; in addition, the stage of the disease and tumor size are the only clinical prognosis factors of adult-type GCTO. Cell atypia, mitotic activity, tumor malignancy degree, and Call-Exner bodies are conventional morphological parameters of GCTO, however, it is commonly impossible to predict relapses from these data [8].

It should be noted that GCTO produce a number of steroid (mainly estrogens, less frequently progesterone and androgens) and peptide (inhibin, Muller's inhibiting substance) hormones, which stimulated studies aimed at the development of test-systems and evaluation of the use of these hormones as the markers of ovarian neoplasms.

It is now established that inhibins are a family of glycoprotein hormones, their level in women of reproductive age depends on the phase of menstrual cycle [10].

N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences; *Department of Reproductive Medicine and Surgery, Postgraduate Education Faculty, Moscow State University of Medicine and Dentistry, Moscow, Russia. ***Address for correspondence:*** biochimia@mtu-net.ru. N. V. Lyubimova

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Apart from Muller's inhibiting substance and activin, inhibins belong to superfamily of β-transforming growth factor. According to their molecular structure, inhibins are glycoproteins consisting of 2 polypeptide chains. Heterodimer formation from α-subunit and one of two β-subunits (β_A and β_B) present in the cell in inactive state is associated with their activation. α-Subunit connected to β_A-subunit via a disulfide bridge corresponds to inhibin A and dimer of α- and β_B-subunits corresponds to inhibin B. Moreover, biological fluids contain free α-subunit not conjugated with β-subunit, a plentiful circulating physiologically inert monomer, and its precursors (pro-α_C, pro-α_N). In total, 6 different molecular forms of inhibin were identified in the dimer form, and only two of them, with a molecular weight of 33 and 66 kDa, possess endocrine as well as endocrine activity.

Ovaries are the main place for inhibin synthesis. This is confirmed by the fact that its levels in sterilized women and in healthy menopausal women are undetectable or extremely low. It is now established that inhibin A is produced in major dominant follicle, while inhibin B is produced in developing antral follicles and its main endocrine effect is feedback regulation of gametogenesis in response to follicle-stimulating hormone production by the pituitary gland. In women of reproductive age, serum concentration of inhibin varies during the menstrual cycle. Secretion of inhibin A peaks in the ovulatory and early lutein phase, whereas inhibin B serum level increases during the early follicular phase, attains maximum in the middle of this phase, slightly decreases by the end of this phase, and further increases during ovulation; during the lutein phase, the concentration of inhibin B gradually decreases to the baseline values corresponding to the detection limit of the available immunoenzyme method [6].

The fact that ovarian follicular epithelium in healthy women is the main source of inhibin prompted studies of its expression in ovarian tumors and in patient serum as a GCTO marker [3]. However, most studies were carried out with investigation of immunoreactive inhibin including inhibin dimers and free α-subunit using available commercial and developed by individual research groups radioimmune, immunofluorimetrical and immunoenzyme test-systems [4,5,9]. Only few studies focused on investigation of the role of inhibin B in diagnostics and monitoring of GCTO, which can be explained by certain methodological difficulties and later appearance of standardized test-systems on the basis of two highly specific antibodies with high analytical sensitivity to inhibin B dimers and low cross-reactivity with its α-subunit present in the blood in the monomer form [7]. We should also note the absence of standardized normal value for inhibin B for interpretation of the results of hormone level evaluation in patients with ovarian neoplasms. This issue is complicated due to variability of inhibin and methodical approaches for its investigation.

The objective of this study was investigation of clinical significance of inhibin B at the time of relapse of adult-type GCTO.

MATERIALS AND METHODS

The study included 33 women with adult-type GCTO, patients of N. N. Blokhin Russian Oncological Center, Russian Academy of Medical Sciences, during the period from January 2005 to March 2010 (5 patients aged 28-57 years with primary GCTO, 19 patients at the age 18-68 years with relapses, and 9 patients aged 23-56 years without evidences for disease progression). In all patients with GCTO relapses, the primary surgery always included castration. The tumor was diagnosed on the basis of histological analysis of the postoperative material. The control group included 20 healthy women: 10 women aged 24-47 years with unaffected menstrual cycle and 10 women in menopause (46-70 years). The reference groups included 9 patients with benign ovarian tumors aged 17-68 years and 5 patients with ovarian cancer (22-67 years).

Evaluation of inhibin B serum level was carried out using standard ACTIVE Inhibin B ELISA kit (Diagnostic System Laboratories). This test-system is based on direct two-stage ELISA with double highly specific monoclonal antibodies to inhibin α- and β_B-subunits and employs the streptavidin technology, which allows detection of dimer inhibin B only. Detection was carried out using universal Elx 800 reader (BIO-TEK INSTRUMENTS).

The data were processed using Kruskal−Wallis test and Spearman correlation test. Threshold values were evaluated using ROC-analysis. Differences of frequencies between the groups were assessed using nonparametric χ^{1-2} test. Differences were considered significant at $p<0.05$.

RESULTS

Taking into account the relationship between serum concentration of inhibin B and phase of the menstrual cycle in women of reproductive age, as well as the presence of young women (18-45 years) with unaffected menstrual function in the examined group of patients with GCTO, we first analyzed the level of inhibin B in the control group of virtually healthy women to standardize conditions of investigation in groups of patients. Variability of hormone concentration was minimal in menopausal women: in 10 of 20 virtually healthy menopausal women inhibin B concentration

did not exceed the detection limit (6 pg/ml) and the median was zero (Table 1). However, in women with preserved menstrual function this parameter greatly varied. The highest levels were detected during the follicular phase: 18.4-115.8 pg/ml, median 47.1 pg/ml. In the middle of the cycle, inhibin B median was substantially lower (4.6 pg/ml) and at the end of lutein phase it corresponded to zero with minimal individual values (0-6 pg/ml).

Similar results were obtained in control patients with benign ovarian tumors aged 17-68 years with minimal inhibin B levels detected during the lutein phase of menstrual cycle. These data attest to a relationship between inhibin B serum levels and phase of menstrual cycle with typical decrease of its secretion almost to zero by the end of the lutein phase and in menopause. These results are in line with specified detection limit of the test-system for inhibin B detection (6.0 pg/ml): maximal hormone concentrations calculated using calibration curve in virtually healthy menopause women and at the end of the lutein phase of menstrual cycle were equal or below 6.0 pg/ml, which attested to the absence of its secretion. This relationship provides the basis for recommendations for inhibin B measurements in patients with GCTO and preserved menstrual function at the end of lutein phase using threshold level of 6.0 pg/ml.

In accordance with presented data, assessment of inhibin B secretion in patients with GCTO was carried out at the end of menstrual cycle (days 26-30), when inhibin B production by ovarian epithelium drops to zero. Marked variability of inhibin B levels was observed in patients with primary GCTO and relapses (Table 2); median values were high: 188.1 and 300 pg/ml, respectively. At the same time in patients with remission, median corresponded to zero and variability was minimal. Similar results were obtained in the reference group (patients with ovarian cancer), where no inhibin B production was detected. It should be noted that inhibin B concentration in patients with relapses and primary GCTO was significantly higher than in the group of patients in remission ($p<0.05$). Analysis of the relationship between inhibin B levels and number of relapses revealed no differences in this parameter between the groups of patients with first, second or third relapse; despite different intensity of hormone secretion, median values were similar (188.1, 214, and 188 pg/ml, respectively). Diagnostic significance of inhibin B was analyzed with consideration for recommended threshold level (6.0 pg/ml); in all groups of patients with relapses or primary GCTO, the incidence of high hormone concentration was high. Inhibin B levels exceeding the threshold value were detected in 80 and 85% cases in groups of patients with primary tumor and first GCTO relapse, respectively, and in all patients with second and third relapses. At the same time, inhibin B level in patients without GCTO signs and in patients with benign tumors and ovarian cancer did not surpass the threshold values.

TABLE 1. Serum Concentration of Inhibin B (pg/ml) in Virtually Healthy Women with Benign Ovarian Tumors during Menstrual Cycle

Menstrual cycle day	Healthy women (*N*=20)	Patients with benign ovarian tumors (*N*=9)
5-7	47.1 (18.4-115.8)	35 (32.7-47.0)
12-16	4.6 (0-91.1)	–
26-28	0 (0-6)	0 (0-1.6)
Postmenopause	0 (0-5.8)	0 (0-4.1)

Note. Here and in Table 2: inhibin B concentrations are presented as median and range of values.

The presented data demonstrate high diagnostic sensitivity (80-100%) and 100% specificity of inhibin B. This conclusion was confirmed by analysis of the hormone level in patients with GCTO in dynamics. Inhibin B was the first sign of GCTO relapse in 28% cases (7 women of 25), in addition, its increased concentration were detected 2-13 month earlier than clinical relapse appeared (Table 2). Using inhibin B detection, GCTO relapse was revealed earlier (median 5.9 months) and at its lower concentrations (14.5-600 pg/ml, median 89), than using instrumental

TABLE 2. Serum Concentration of Inhibin B (pg/ml) in Patients with Ovarian Cancer and GCTO

Group of patients	Inhibin B concentration
Primary GCTO (*N*=5)	300* (0-488)
Relapse GCTO (*N*=25)	188.1* (0-3000)
Without signs of the disease (*N*=9)	0 (0-2.2)
Ovarian cancer (*N*=5)	0 (0-2)

Note. *$p<0.05$ compared to patients without signs of the disease.

methods of investigation (median for relapse detection 9 months at hormone levels of 37-2200 pg/ml, median 188).

Thus, serum concentration of inhibin B in patients with GCTO relapse was significantly higher than in patients without signs of the disease and in virtually healthy women, when hormone evaluation was carried out at the end of menstrual cycle. The increase in inhibin B concentration was observed 2-13 months earlier than clinical manifestation of disease, which attests to high diagnostic sensitivity of this marker and allows its recommendation for the use in diagnostics and monitoring of GCTO. Inhibin B assay in blood serum together with over clinical and morphological parameters is important for primary and differential GCTO diagnostics, individual choice of treatment strategies in this patient category, assessment of prognosis, and early detection of relapses. Our study showed that inhibin B is highly sensitive and specific GCTO marker, particularly during the preclinical period of relapse development.

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