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MUTATIONS IN BRCA1/2 GENES IN PATIENTS OF SOUTHERN RUSSIA WITH MALIGNANT OVARIAN TUMORS

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The article presents the results of a study of the spectrum of mutations in the BRCA1 / 2 genes associated with the development of hereditary breast and ovarian cancer in patients of South Russia with malignant ovarian neoplasms.

Mutations in the BRCA1 gene were determined by real-time PCR: 185delAG, 300T> C, 2080delA, 4153delA, 5382insC, 3819delGTAAA, 3875delGTCT; in the BRCA2 gene - 6174delT in 178 patients with a histologically verified diagnosis of ovarian cancer.

The study included epithelial tumors (malignant) - 98.1%, and granulosa cell tumors - 1.9%. Of the epithelial tumors, the most common was high-grade serous carcinoma (78%). Based on the results of genotyping, the prevalence of germline mutations in the BRCA1 / 2 genes was revealed at 20.8%. The higher rate of genetic changes is obviously associated with hereditary history (40% of patients). Of the seven identified mutations, 5382insC (67.6%) was revealed more frequently. All patients confirmed the same mutation in the tumor. There were no cases of somatic changes in BRCA1 / 2. The prevalence of BRCA1 mutations was noted in the group of patients with low-grade serous carcinoma, in which all cases of mutations in the BRCA2 gene were identified.

Thus, in patients with OC living in the south of Russia, the mutation frequency in the BRCA1 / 2 genes was 20.8%. The distribution of mutation types with predominance of 5382insC BRCA1 (67.6%) corresponds to the ratio of their occurrence in populations of European countries. BRCA1 / 2 mutations were recorded more frequently in the group of patients with high-grade serous carcinoma.

Keywords: ovarian cancer, mutations, BRCA1 / 2, PCR.

Introduction. Ovarian cancer (OC) is a malignant tumor and the first leading cause of death among gynecologic cancers due to a large number of patients with advanced disease characterized by metastases into the abdominal cavity. About 10-15% of ovarian tumors are associated with hereditary diseases, and about 65-85% of patients with hereditary ovarian carcinomas have a mutation in the BRCA1 and BRCA2 genes involved in DNA repairing and genomic stability maintenance [16]. The lifetime risk of OC developing in women with mutations in the BRCA1 or BRCA2 gene is 35-70% and 10-30%, respectively [16]. The total lifetime risk of developing breast cancer and OC increases to 85% and 60%, respectively, in carriers of BRCA1 and BRCA2 mutations [8]. Hereditary OC was reported to be associated with the presence of gene aberrations related to other hereditary syndromes (TP53 mutations in Li-Fraumeni syndrome, mismatch repair genes (MMR) in Lynch syndrome, double-strand break repair genes BARD1, CHEK2, RAD51, and PALB2) [17].

Aim of the study. The aim of the study was to analyze the spectrum of mutations in the BRCA1/2 genes associated with the development of hereditary breast cancer and ovarian cancer in patients with ovarian cancer in the South of Russia.

Material and methods. The study included 178 women aged 27-71 years old diagnosed with ovarian cancer T1-4N0-1M0-1, gr. 2 (stage I-IV), who received treatment in National Medical Research Centre for Oncology in 2015-2019. The survey revealed one or more risk factors in 71 cases: the age of onset up to 45 years, multiple tumors, bilateral lesions, and hereditary burden. All patients gave their informed consent to the processing of their personal and medical data, as well as to the use of biological material. EDTA venous blood and paraffin-embedded ovarian tissue blocks were studied. Genomic DNA was isolated from whole blood leukocytes and tumor samples using the DNA-sorb-B kit (AmpliSens, Russia). For DNA isolation from paraffin blocks (tissue samples fixed in 10% buffered formalin), 5-8 slices 3 μm thick were obtained using a microtome, dewaxed with o-xylene and 95% ethyl alcohol, lysed overnight in 200 μl of lysis solution with the addition of 20 μl of

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proteinase K (10 mg/ml) at 58°C, then heated at 90°C for 1 hour to eliminate DNA-protein cross-links, and processed using the DNA-sorb-B kit according to the instructions. Mutations were detected by real-time PCR using the OncoGenetics BRCA kit (DNA-Technology, Russia). Mutations were detected in all patients in the BRCA1 gene: 185delAG, 300T>C, 2080delA, 4153delA, 5382insC, 3819delGTAAA, 3875delGTCT; in BRCA2 – 6174delT. The registration and interpretation of the reaction results was performed automatically using the software supplied with the detecting amplifier DTprime 5M1 (OOO NPO DNA-Technology, Russia).

Statistical analysis of the results was performed using applied statistical programs Microsoft Excel 2013 (Microsoft Corporation, USA) and STATISTICA 8.0 (Stat Soft Inc., USA). Differences were evaluated with the nonparametric Mann-Whitney U-test; the frequencies of characteristics were compared using the χ^2 test.

Results and discussion. The data on genotyping of DNA extracted from the blood and tumor tissue were obtained for all 178 patients. Differences between the mutation statuses in the blood and tumor material were not registered. Table 1 presents the distribution of BRCA1/2 mutations among patients of various age groups.

Analysis of OC patients' age demonstrated that the mean age of the disease onset in wild-type BRCA1/2 was 52.4 years, for the mutant type – 54 years. As a result, no differences were found in the age of onset between two groups depending on the status of the BRCA1/2 gene (U=-0.133 at p=0.894). However, distribution of patients with different mutation statuses by age groups revealed statistically significant differences (χ^2=18.47 at p<0.01). Mutations were most frequent in patients aged 45-64 years old, while in elder women (>65 years) wild-type BRCA1/2 prevailed (Table 1).

Mutations in the BRCA1/2 genes were found in 37 (20.8%) OC patients. Germline mutations in the BRCA1 gene were detected in 35 women, in BRCA2 – in 2 women. Six out of eight studied mutation types were found in OC patients (Table 2). BRCA1 5382insC mutation was the most frequent (67.6%).

OC is a heterogenic disease with morphological as well as molecular genetic features. Most OC types are classified as epithelial (90%), with histological subtypes such as serous (68-71%), endometrioid (9-11%), clear cell (12-13%), mucinous (3%), malignant Brenner tumors (1 %) and mixed carcinomas (6%) [12]. Depending on the genetic profile, serous carcinomas are divided into clearly distinguishable serous carcinomas with low malignant potential including lesions associated with borderline ovarian tumors and G1 adenocarcinomas (genetic type I), and barely distinguishable serous carcinomas with high malignant potential including G2 and G3 tumors (genetic type II) [1, 2].

In this study, epithelial (malignant) tumors were histologically verified in 98.1% cases, and adult granulosa cell tumors – in 1.9%. Barely distinguishable serous carcinoma was the most frequent epithelial tumor (78%).

The main proportion of mutations in the BRCA1 gene was observed in the largest group of high-grade serous carcinomas (67.6%), and mutations in the BRCA2 gene were identified only in this group (Table 2). However, no statistically significant differences in mutational status were found between different histological subtypes (Table 3).

The BRCA1/2 genes play an important role in maintaining DNA integrity by participating in the repair of double-strand breaks by homologous recombination, which, when disrupted, is responsible for the accumulation of genomic changes and eventual genomic instability [4]. The identification of pathogenic mutations allowed determining the mechanisms of the pathology development, as well as predicting the risks for carriers of germline mutations. While the general population risk of ovarian cancer averages 1-2%, it increases in families with mutations identified in these genes to 39-63% for BRCA1 and 16-27% for BRCA2 [8].

In this study, mutations in the BRCA1/2 genes were detected in 20.8% of OC patients. An increased rate of genetic changes was apparently associated with a large number of patients with hereditary burden (40%). All people with pathogenic BRCA1/2 mutations were referred for a genetic counseling to form the groups with high risks of breast cancer, OC and other tumors, including pancreatic [14,

Table1

Status of the *BRCA1/2* genes in various age groups of OC patients

Age group*	Wild type (%)	Mutant type (%)
<34	11.5	0
35-44	19.2	11.1
45-54	15.4	44.4
55-64	23.1	29.6
>65	30.8	14.8
χ^2 (p)	18.47 (<0.01)	

Note. *Here and in Tables 2 and 3: the age at initial diagnosis is given.

Table2

Rates of genotypes with a mutant allele of the *BRCA1/2* genes in patients with different histological types of OC.

Studied mutations		Barely distinguishable serous carcinoma (%)	Clearly distinguishable serous carcinoma (%)	Mixed epithelial tumors (%)
BRCA1	185delAG	2.7 (1/37)	0	0
	4153delA	13.5 (5/37)	0	0
	5382insC	41.1 (15/37)	13.5 (5/37)	13.5 (5/37)
	3819delGTAAA	0	0	0
	3875delGTCT	0	0	0
	300T>C	2.7 (1/37)	0	0
	2080delA	8.1 (3/37)	0	0
BRCA2	6174delT	5.4 (2/37)	0	0

Table3

Status of the BRCA1/2 genes in different histological types of OC

Histological subtype of OC	Wild type (%)	Mutant type (%)
Barely distinguishable serous carcinoma	50.6	15.2
Clearly distinguishable serous carcinoma	15.2	2.8
Mucinous tumors	4.5	0
Endometrioid tumors	6.2	0
Mixed epithelial tumors	1.7	2.8
Adult granulosa cell tumor	1.1	0
Total	79.2	20.8
χ^2 (p)	9.377 (>0.05)	

6] and prostate [11] cancer. 2 patients (5.4%) developed other tumors during clinical observation.

Results of BRCA genetic tests are important for OC patients, for example, as a prognostic biomarker of response to specific antitumor therapy [5]. Studies have shown that patients with a positive test for pathogenic BRCA1/2 variants demonstrate higher sensitivity to combination therapy with platinum derivatives, and higher sensitivity to treatment with PARP inhibitors compared to patients without such variants [5]. Inability to repair chemotherapy-induced DNA damage results in significantly better prognosis in BRCA-positive patients with progressing disease compared to wild-type patients. BRCA-positive OC patients are at risk of developing secondary cancers. BRCA-positive test in patients with OC is also important for assessing the risk of cancer development and its prevention among relatives [5].

Recent studies showed that the prevalence of hereditary pathogenic variants and assessment of gene-specific risk may vary based on family history and type/molecular subtype of tumors, as well as on the race, ethnicity and geographic place of residence [3, 7, 13]. In this study, rates of the BRCA1 gene mutations (Table 2) generally corresponded to that for European countries, and the most common mutation 5382insC occurred with the same frequency as in the previously published data (67.6% vs 68.3%) [10].

Conclusion. Thus, the study demonstrated that the frequency of mutations in the BRCA1/2 genes in OC patients living in the South of Russia was 20.8%. The distribution of mutation types with a predominance of 5382insC BRCA1 (67.6%) corresponded to their rates in populations of European countries. Carriers of mutations in the BRCA1/2 genes were more often observed in the group of patients aged 45-64 years old with barely distinguishable serous carcinoma.

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