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BREAST CANCER IMMUNOHISTOCHEMICAL FEATURES IN YOUNG WOMEN WITH *BRCA1/2* MUTATIONS

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Breast cancer among young women is a rare pathology. In most studies published so far, this patients group is not being analyzed separately. Particularities of this pathology require an additional examination of the immunohistochemical and molecular-genetic markers of the disease for development of the effective treatment protocols. The mutations of *BRCA1/2* are the important factor impacting to the disease prognosis along the age of the patient. *Aim:* To compare the expression of prognostically meaningful immunohistochemical markers such as estrogen receptor (ER), progesterone receptor (PR), HER-2, p53, Ki-67, in tumor cells of the female patients with breast cancer aged less than 36 depending on the presence or absence of mutations in *BRCA1/2* genes. *Methods:* Two hundred forty-eight patients aged less than 36 at the time of diagnosis of breast cancer were examined clinically. Expression of ER, PR, HER-2, p53, Ki-67 was determined by indirect immunohistochemical method. Mutations of *BRCA1* (185delAG, 5382insC) and *BRCA2* (6174delT) genes were screened using multiplex PCR in 99 patients. *Results:* Mutations of *BRCA1/2* genes were found in 9.1% of patients. More aggressive clinical course of the disease was seen in mutations carriers, who had 3-years survival of only 55.6%. They did not demonstrated expression of ER, PR, and HER-2 in 88.9% of the cases. Whereas, patients without *BRCA1/2* mutations did not express ER, PR, and HER-2 in only 42.0% of cases. There were no differences between patients with and without mutations in terms of tumor size, presence of metastases in lymph nodes, p53 and Ki-67 expression. *Conclusion:* Presence of *BRCA1/2* genes mutations in young women is associated with more aggressiveness of the breast cancer (their 3-years survival is 25, 5% less) and absence of the ER, PR, HER-2 receptors, which is unfavorable prognostic factor in terms of hormone therapy. These data should be taken into account at chemotherapy planning, especially in patients with early stages of the disease. There were no differences between patients with and without *BRCA1/2* mutations in terms of tumor size, lymph nodes involvement, tumor histology, and p53 and Ki-67 proteins expression.

Key Words: **breast cancer, young patients, *BRCA1/2* gene mutations, immunohistochemical markers.**

Breast cancer is one of the most common malignant tumors and a main cause of death from oncological diseases in women worldwide. One third, and in economically developed countries up to 40% of all cases of breast cancer is diagnosed in female patients older than 65 years [1]. Young patients account from 2 to 5% of all breast cancer population [2–4]. In Ukraine the incidence of women for breast cancer increases with the age of the patients, reaching maximum in age group of 70–74 (148.9 per 100 000 of female population), then decreases in age groups of 75–79, 80–84, 85 years and older where accounts 113.2, 108.5 and 74.3 per 100 000 of female population respectively. The rate of morbidity for breast cancer in age groups of 15–19 years, 20–24 years, 25–29 years, 30–34 years is 0.1, 1.3, 3.2 and 13.7 per 100 000 of female population accordingly [3]. As this pathology is rare in young women, there are limited studies published so far on early breast cancer. Most of them are based on the data obtained from the retrospective studies on the small number of cases [2, 4].

The early breast cancer is often characterized by the presence of a low histological differentiation, high mitotic activity, and frequent vascular wall invasion. There is no common opinion regarding the age peculiarities of Her2/neu, p53, progesterone receptor expression [5–8]. There are controversial data on tumor sizes and lymph nodes involvement characteristics in patients with early breast cancer. M. Colleoni *et al*. [9] did not observe the bigger size of tumor and lymph nodes involvement in young female patient in contrast to the opinion of a numerous authors who reported it [7, 10]. W. Lee *et al*. [11] demonstrated the absence of differences in Her2/neu and p53 expression in cells of breast cancer female patients in young and elderly age.

Many investigators pointed out the shorter three- and five-years survival of young female patients in comparison with elderly females in premenopausal and postmenopausal age. This was the reason to include the age into the list of negative prognostic factors in breast cancer. Young female patients (35 years old and younger) need more chemotherapy compared to the older patients according to recommendations of St Gallen consensus.

There are a lot of studies demonstrated that cancer patient's survival depends not only on the stage of the disease, but also on the intrinsic biological factors, which predispose the characteristics of the tumors. The key neoplastic event is the genetic change, which leads to uncontrolled cell growth, loss of ability to differentiate and disturbance of apoptosis. Situation with the molecular-genetic features of the early breast cancer is not clear. The changes in estrogen receptor (ER), progesterone receptor (PR), HER-2/neu

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Abbreviations used: ER – estrogene receptor; PCR – polymerase chain reaction; PR – progesterone receptor.

expression, presence of apoptotic markers, changes of indices of proliferative activity are demonstrated for breast cancer patients of young age [12]. However, in spite these characteristics have the clinical prognostic value, the protocols of early breast cancer diagnostics and treatment urgently need an improvement. A lot of young breast cancer patients received too aggressive therapy, whereas, some of them are not effectively treated and died prematurely. It is very important to have the objective molecular-biologic criteria, which could classify the aggressiveness of cancer in young patients. The *BRCA1/2* mutations are very common in young breast cancer patients. Thus, it could be assumed that they play an important role in carcinogenesis and predetermine the prognosis [13]. There are numerous studies linked *BRCA1/2* mutations with unfavorable clinical prognosis [14, 15].

BRCA1 (OMIM 113705) and *BRCA2* (OMIM 600185) genes are associated with the prevalence of hereditary forms of breast cancer and ovary cancer. The presence of germinal mutations of *BRCA1* and *BRCA2* genes increases the risk of the disease in women aged 50 by 30–50% and in women of 80 years old by 60–90% [16–18]. The spectrum and frequency of mutations in *BRCA1* and *BRCA2* genes differs in various ethnic groups, therefore, special investigations are needed for each population [19–25].

Pathogenesis of *BRCA*-associated and sporadic breast cancer have a lot of differences. It has been demonstrated that the size of the breast tumor in carriers of *BRCA1/2* mutations is greater and the absence of regional lymph nodes involvement does not lead to the decrease of mortality in carriers of mutated genes. Tumors associated with pathologic *BRCA*-genotype have a short duplication period, higher malignancy level. According to the majority authors' data, 64–92% of tumor cases associated with germinal mutations of *BRCA1/2* genes do not have receptors of estrogen and progesterone; although in some cases patients with *BRCA2* mutations have paradoxically high expression of receptors of steroid hormones. One might consider germinal mutations of BRCA genes as molecular-genetic markers that have a prognostic value. The development of diagnostic algorithm needs receiving sufficient data for selecting the most effective treatment regimen for young breast cancer female patients. Immunohistochemical and genetic investigations methods acquire a particular value currently. The most authors report that patients with BRCA1/2 mutations have bigger tumor size, more often have metastasis in lymph nodes and rarely express ER and PR [26]. The data on Her-2, p53, Ki-67 in BRCA1/2 carriers are controversial.

MATERIALS AND METHODS

The investigation was carried out on 248 retrospective and prospective cases of breast cancer in women younger 36 years of age who had been treated at the Kiev City Oncological Hospital through 1997–2007, which accounted 3.2% of all hospital patients suffering from this pathology. All patients provided written consent to perform the study.

In majority of cases, tumor size was small: T1 — 142 cases (57.3%), T2 — 85 cases (34.3%), T3 — 16 cases (6.5%), and T4 — 5 cases (2.0%). Initial disease stages were seen more often: I stage (T1-2N0M0) — 46.8%, II stage (T1-2N0-1M0) — 43.2%. In 127 cases patients underwent mastectomy (51.21%), in 83 cases — tissue-sparing operation (33.5%), and 38 patients underwent only conservative treatment (15.3%). Verification of the diagnosis was done by histology on the core biopsy material. In the majority of cases, tumor was diagnosed as an infiltrating ductal carcinoma — 233 cases (93.95%), in some cases — infiltrating lobular carcinoma (8 cases), medullar (3 cases), giant cellular (1 case), squamous cell (1 case) carcinoma, carcinosarcoma (1 case), Pedget cancer (1 case).

Paraffin-embedded tumor sections from 99 patients were used for immunohistochemical investigation. Indirect immunohistochemical methods with monoclonal antibodies against estrogen (clone 1D5), progesterone (clone PgR 636), oncoprotein c-erbB-2 (HER-2/neu), p53, Ki-67 and LSAB and EnVision visualization system (DAKO) were used. Results were assessed by calculating the percentage of tumor cells, having stained nucleus with indication of intensivity of staining (from 1+ to 3+). In assessing the results of reaction with c-erbB-2 antibody we registered the presence of intensive membrane staining of the cell (from 1+ to 3+). Tumors with detected staining intensivity 2+ and 3+ were considered to be positive.

In 99 from 248 young patients the mutations in *BRCA1/2* genes (mutations 5382ins and 185delAG in *BRCA1* gene, and mutation 6174delT in *BRCA2* gene) were screened using DNA isolated from peripheral blood or from formalin fixed paraffin-embedded tumor tissue sections if the patient was dead at the time of cases recruitment. DNA isolation from paraffin-embedded sections was performed using method suggested by J.L. Bernstein *et al*. [27]. DNA isolation from peripheral blood was performed by a standard method using a commercial test-system DNA Sorb B kit (AmpliSens).

The conditions of DNA amplification and primers sequence for detecting of mutations 5382ins and 185delAG in *BRCA1* gene, 6174delT in *BRCA2* gene were published earlier by P.C. Chan *et al*. [28].

RESULTS AND DISCUSSION

Mutations of *BRCA1/2* have been detected in 9 female patients from cohort of 99 breast cancer young patients (9.1%). Mutation 185delAG in *BRCA1* gene was detected in 1 patient, mutation 5382ins in *BRCA1* gene — in 7 patients, mutation 6174delT in *BRCA2* gene — in 1 patient (Table 1). During the first year after making the diagnosis, 4 patients from 9 died (44.4%). The other 5 young women lived over 3 years, and 3 of them lived over 5 years after surgical intervention. Thus, 3-years survival reached 55.6%. In the group of living patients, T1N0M0 was diagnosed in 3 cased, and T2N1M0 — in one case. The size of tumor corresponding to T1 had

44.4% patients with mutations of *BRCA1/2* genes, T2 — 34%, T3 — 21.5%. Lymph nodes metastases were detected in 33.3% of patients. One of the died patient of this group had the I stage of disease (T1N0M0), the second one — II stage without lymph nodes involvement (T2N0M0) and only 2 patients had metastases in lymph nodes (T2N1M0 и T3N1M0). Thus, probably, aggressive course of the disease in those women is not predetermined by the advanced stage of the disease at the moment of diagnosis but rather by biology of tumor.

Table 1. The results of immunohistochemical studies of young women with breast cancer and *BRCA1/2* mutations

Case	TNM	Type	Grade	Immunohistochemical staining					Survival rate
				ER	PR	Her-2/neu	Ki-67	p53	
Mutation 5382 insC in BRCA1gene									
1	T1N0M0	Ductal carcinoma	I	Neg	Neg	Neg	90%	60%	Alive 6 years
2	T2N1M0	Lobular carcinoma	II	Neg	Neg	Neg	23%	20%	Alive 10 years
3	T1N0M0	Ductal carcinoma	II	Neg	Neg	Neg	70%	70%	Alive 3 years
4	T1N0M0	Ductal carcinoma	I	Pos	Pos	Neg	40%	20%	Alive 5 years
5	T1N0M0	Ductal carcinoma	II	Neg	Neg	Neg	68%	70%	Lived less than 1 year
6	T2N0M0	Ductal carcinoma	II	Neg	Neg	Pos	53%	100%	Alive 4 years
7	T3N1M0	Ductal carcinoma	II	Neg	Neg	Pos	75%	100%	Lived less than 1 year
Mutation 185 del AG in BRCA1 gene									
8	T2N1M0	Ductal carcinoma	III	Neg	Neg	Neg	60%	100%	Lived less than 1 year
Mutation 6174 del T in BRCA2 gene									
9	T3N0M0	Ductal carcinoma	III	Neg	Neg	Pos	40%	90%	Lived less than 1 year

Notes: In Tables 1, 2, 3: Neg – negative; Pos – positive.

In the group of young age patients without mutations in *BRCA1/2* genes small size of tumors was predominant: T1 in 46.6% of cases, T2 — in 44.4%, T3 — in 8.8%, T4 — in 0.2%. More than half of the patients (51.6%) did not have metastases in lymph nodes and 46% of patients had metastases lesions in only one group of lymph nodes, 2.4% — in two groups. 81.1% of female patients lived 3 and more years. No differences in histological cancer variants in women with mutations of *BRCA1/2* and without mutations were found.

Therefore, in cases of similar disease stages and similar histological cancer types, the clinical course of breast cancer in young age patients having *BRCA1/2* genes mutations was more aggressive. The index of 3-years survival of patients with *BRCA1/2* genes mutations was lower by 25.6%.

Estrogen and progesterone receptors expression was seen only in one case out of 9 (11.1%) patients with mutations in *BRCA1/2* genes (Fig. 1, 2), whereas in patients without mutations 57.97% of the cases have an estrogen receptor ($p < 0.05$) and 56.52% of the cases have a progesterone receptor ($p < 0.05$) (Table 2, 3).

Table 2. ER and PR status in women with and without *BRCA1/2* mutations (mean)

		Young women < 36 years		
		With *BRCA1/2* mutations	Without *BRCA1/2* mutations	All young women
ER	Pos	11.11%	57.97%	49.3%
PR	Pos	11.11%	56.52%	47.3%
	Neg	88.89%	43.48%	52.7%

Table 3. Estrogen (ER) and progesterone receptor (PgR) status in women with and without BRCA1/2 mutations

Immunohistochemical staining	Results	Young women < 36 years		
		With *BRCA1/2* mutations, %	Without *BRCA1/2* mutations, %	All young women, %
ER	Pos	8.3 ± 7.9	41.4 ± 4.7	38.2 ± 4.4
PR	Pos	8.3 ± 7.9	44.1 ±4 .7	40.6 ± 4.4
HER-2	Pos	16.7 ± 10.8	6.3 ± 2.3	7.3 ± 2.3
p 53	> 40%	58.3 ± 14.2	15.3 ± 3.4	19.5 ± 3.6
Ki-67	> 40%	50 ± 14.4	16.2 ± 3.5	19.5 ± 3.6

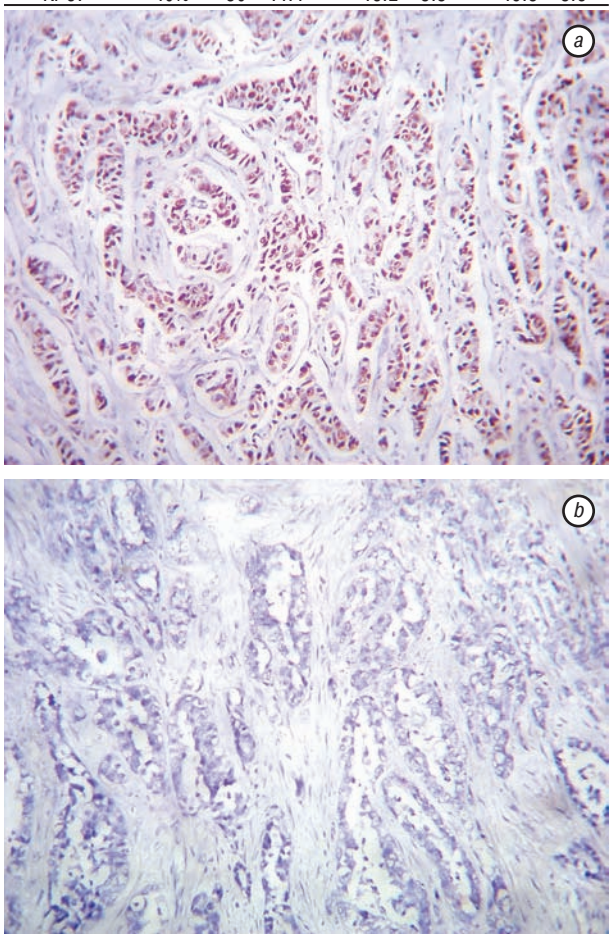

Fig. 1. Estrogen receptor expression by breast tumor cells. *a*, Estrogen receptor has a nuclear expression in the majority of the tumor cells. *b*, Case with negative estrogen receptor expression. Immunohistochemistry staining, visualisation system EnVision, chromogene DAB, X 400

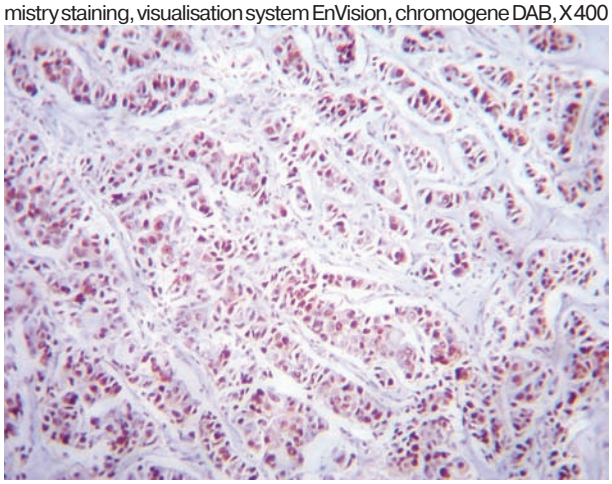

Fig. 2. Progesteron receptor has a nuclear expression in the majority of the tumor cells. Immunohistochemistry staining, visualisation system EnVision, chromogene DAB, X 400

Overexpression of oncoprotein HER2/neu was observed in 3 cases from 9 in patients with mutation of *BRCA1/2* genes (33.3%) and in 13.85% in breast can-

cer women without mutations of those genes (Fig. 3). Protein p53 expression in more than 50% of tumors cells was observed in 6 cases from 9 in case of the presence of *BRCA1/2* genes mutation (66.7%) and in 50% of female patients without mutations of those genes (Fig. 4). Mitotic tumor activity in patients of the both groups was insignificantly different: high mitotic index 57.1% was in mutated breast cancer women and in 50% cases in unmutated breast cancer patients (Fig. 5).

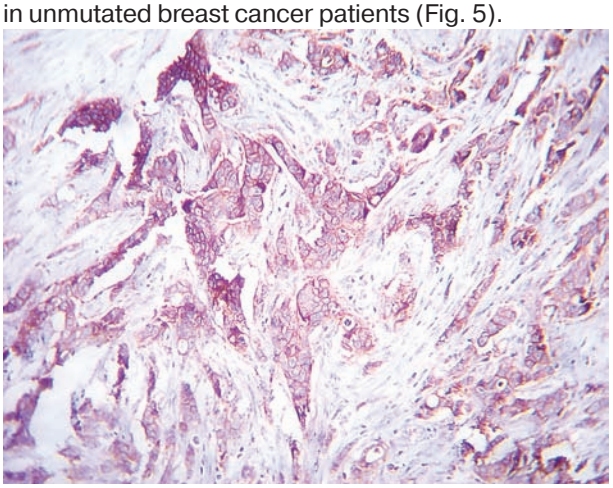

Fig. 3. HER-2/neu (C-erB-2 oncoprotein) (2+) membrane expression in the tumor cells. Immunohistochemistry staining, visualisation system EnVision, chromogene DAB, X 400

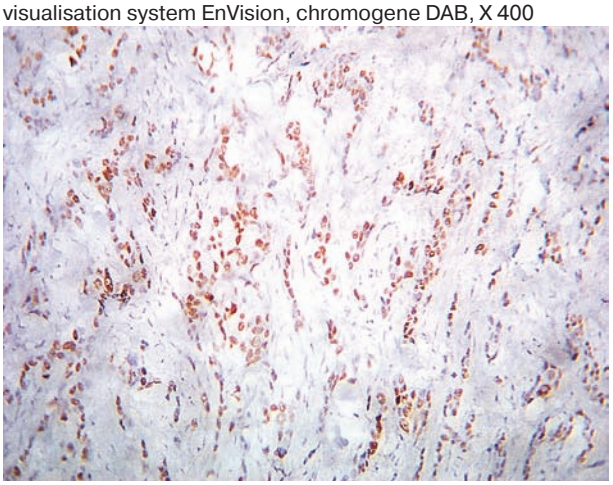

Fig. 4. P53 protein was expressed by all tumor cells. Immunohistochemistry staining, visualisation system EnVision, chromogene DAB, X 400

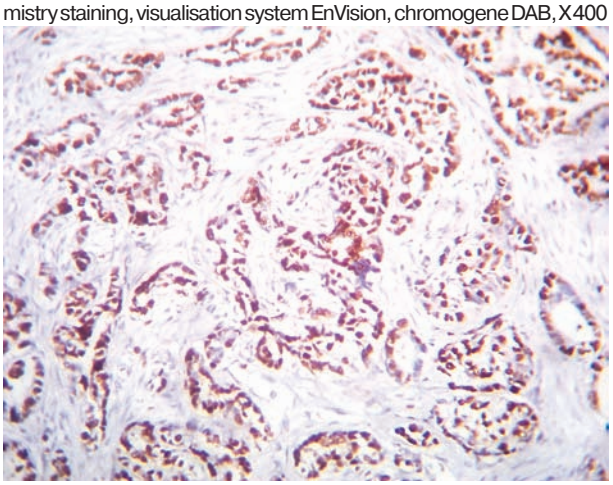

Fig. 5. Ki-67 protein was expressed in 70% of the tumor cells. Immunohistochemistry staining, visualisation system EnVision, chromogene DAB, X 400

In conclusion, *BRCA1/2* genes mutations were found in 9.1% of the patients. No differences in histological cancer variants and disease stages in women with mutations of *BRCA1/2* and without mutations were found. The presence of mutations in *BRCA1/2* genes in young women is associated with more aggressive course of the breast cancer and absence of the progesterone and estrogene receptors (see Fig. 1), which is unfavorable prognostic factor.

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