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IMMUNOHISTOCHEMICAL AND BIOCHEMICAL ANALYSIS OF MAMMARY GLAND TUMOURS OF DIFFERENT AGE PATIENTS

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Immunohistochemical and biochemical study of infiltrative ductal breast carcinoma and tissue adjacent to the tumour revealed a particular molecular profile and characteristics of the oxidant-antioxidant status neoplasms depending on the age of the patients and the presence of metastases in regional lymph nodes. Some causes of high aggressiveness and low hormone sensitivity of tumours in premenopausal women, as well as stability and high metastatic potential of tumours in postmenopausal women have been found.

Key words: *breast tumours, the molecular profile, oxidant-antioxidant characteristics, metastasis, menopausal status.*

Introduction. Breast cancer (BC) is the most common cancer and the second leading cause of cancer death in the world. It is a leader in cancer incidence structure of the female population in the most economically developed countries. For example, it was estimated that almost 1.7 million cases of female breast cancer were diagnosed worldwide, corresponding to a rate of 43 per 100,000 and about 522,000 females (13 per 100,000 populations) have died from breast cancer globally during 2012. Deaths of women from breast cancer has increased more than 2.5 times over the past 10 years and won the first place [1, 2]. The problem of the incidence and treatment of malignant tumours of the breast, as well as disability of population as a result of this disease continues to merit serious consideration, since the number of the patients increases, and the results of treatment, medical and social rehabilitation cannot be considered sufficiently satisfactory. This causes the relevance and importance of finding and developing new methods for anticancer treatment.

Survival from breast cancer depends mainly on early detection and optimal treatment. Basic knowledge on the mechanisms causing breast cancer progression has driven significant progress in its treatment, with the introduction of more sophisticated hormonal and targeted therapies [3]. Despite advances in early detection and understanding of the molecular bases of breast cancer biology, approximately 30 % of all patients with early-stage breast cancer have recurrent disease, which is metastatic in most cases. Breast cancer treatment currently requires the joint efforts of a multidisciplinary team. Developing such a plan should be based on knowledge of the benefits and potential acute and late toxic effects of each of the therapy regimens. Tumour biology plays important role in breast cancer survival. To offer more effective and less toxic treatment, selecting therapies requires considering the patient and the clinical and molecular characteristics of the tumour.

There are differences in histological types of breast cancer as well as the molecular profile of breast cancer. Modern approaches to the treatment of patients with breast cancer suggest an individualized choice of the type of treatment based on the risk category and the sensitivity of tumour cells to the hormone. To date, the choice of adjuvant therapy is dependent on the extent of the primary tumour and its histological grade embodiment, the level of expression of steroid hormone receptors, the reproductive status and age of the patients [4]. However, after a variable period of time, progression occurs. At that point, resistance to therapy is not only common but expected [5].

The role of estrogen receptor (ER) and progesterone receptors (PgR) in the genesis of hormone dependent tumours is proved in many studies. Now determination of ER and PgR in the tumour tissue is considered as a prerequisite for successful hormonal treatment of breast cancer [6, 7]. Degree of response to hormonal therapy is also significantly dependent on the presence of ER and PgR in tumours: its efficiency is about 10 % of ER negative tumours, approximately 50 % of ER positive tumours and 75 % for tumours containing both ER

and PgR [8]. However, after determining the stage, histological grade and hormone receptor status, the tumour can behave in an unexpected manner, no significant relation to receptor status or tumor grade was seen and the prognosis can vary. There are cases of resistance to hormone therapy in patients with ER- and PgR-positive tumours, so hormone receptor status of the tumour is not always a sufficient indicator of the hormonal sensitivity of breast cancer [9, 10].

Leading factor in the mechanism of malignant transformation of cells in the biological behaviour of tumours that have already arisen is their proliferative activity. At breast cancer the most important prognostic indicators are proliferative activity determined on the expression of the protein HER2/neu (c-erbB-2). Membrane glycoprotein HER-2 is one of the most promising markers for the prediction of recurrence of the disease, metastasis, resistance to chemotherapy and hormone therapy of breast cancer [11, 12]. Overexpression of the human epidermal growth factor receptor 2 (HER2) protein, mainly due to HER2/neu gene amplification, in breast cancer is associated with aggressive histological features and poor prognosis [13]. According to many researchers the HER2/neu allows to estimate prognosis of the disease, especially in the presence of metastases in regional lymph nodes. Its overexpression in the tumour (tumour HER2/neu+) is followed by a sharp decrease in apoptosis, increase of cell proliferation, decrease of the amount of estrogen receptors in the tumour, decrease of the effectiveness of chemotherapy and hormonal therapy etc. Under the influence of ligands HER2/neu forms heterodimers with other receptors of this family and regulates the relevant signalling cascades [14, 15]. In the process of malignant growth its overexpression and/or amplification of the gene encoding it takes place. Overexpression of HER2/neu tumour is an independent marker of poor prognosis, increased risk of disease recurrence and reduction of survival. It is established that the oncoprotein c-erbB-2 is overexpressed in 20–30 % of cases of invasive breast cancer [16, 17]. HER2/neu overexpression affects the results of hormone and chemotherapy, and its determination may help in choosing the rational treatment of patients with breast cancer.

To identify the features of cell proliferation of human malignant tumours antigen Ki-67 expressed in virtually all phases of the mitotic cycle and reflecting the magnitude of the proliferative pool is used widely. Index of Ki-67 antibody is a reliable prognostic marker in determining the number of tumour cells that are in the S period of the cell cycle. Antigen Ki-67 is as an independent prognostic factor with regard to recurrence in patients. Ki-67 refers to regulatory proteins. Its appearance coincides with the entry of cells into mitosis; it can be used as a universal proliferation marker in assessing the growth of malignant tumours [18].

The progression of breast cancer is associated with oxidative stress [19, 20]. Reactive oxygen species (ROS) are known to damage cellular macromolecules and to modulate signalling cascades in a variety of human diseases including cancers [21]. Under normal circumstances, there is a steady balance between the production of oxygen derived free radicals and their destruction by the cellular antioxidant system inside the human body. However, oxidant/antioxidant balance has been suggested as an important factor for initiation and progression of cancer: any imbalance between the levels of these oxidants and antioxidants might cause DNA damage and may lead to tumour development [22]. It would seem that oxidative stress induces cell cycle arrest in the breast cancer by modulation of these genes. [23]. Overproduction of ROS triggers estrogen receptor activity [24].

Increasing chronological age is the most significant risk factor for human cancer development [25]. Mortality trends tended to be more favourable for women aged under 50 compared to those who were 50 or older [1]. The relationship between aging and cancer is complex because the interaction takes place at the cell, the organism and the environment levels. On the other hand, carcinogenesis is a multi-step process, and different mechanisms may be involved in each step. The age specificity of some breast risk factors suggests that breast cancer which has been diagnosed in an aged woman was induced late in her life [26]. The difference in tumour aggressiveness between young and older patients is especially obvious in breast cancer patients. Tumours in older patients are generally described as slow growing. For example, oxidants and antioxidants may play different roles depending upon the phase considered. Breast carcinoma is related to the increase of lipid peroxidation in plasma with concomitant decrease of antioxidant (AO) defence

capacity in blood cells, which becomes more pronounced during aging of the patients [27].

The aim of this study was determination of differences in some features of breast tumour depending on the age of the patients and metastasis presence to derive their possible role in the etiology of breast cancer.

Materials and methods. The study was conducted in Dnepropetrovsk. Samples of the breast tumour and unaltered surrounding background breast tissue were obtained from the Regional Cancer Centre. Tissue samples of 88 patients with primary breast cancer were collected during surgery, snap-frozen, and stored in liquid nitrogen. Patients suffering from infiltrative ductal carcinoma of II–III degree of malignancy were divided into two groups. Group I (n = 40) was comprised by patients aged 40–50 years old at diagnosis in premenopausal period (n = 40). This group was divided into two subgroups – IA (n = 27), the patients with the primary tumor, and IB (n = 13), patients with the regional lymph node metastases. Group II (n = 48) was comprised patients aged 50 years and older in postmenopausal period: subgroups – IIA (n = 40), patients without metastases in regional lymph nodes, and IIB (n = 8), patients with the metastases. The patients included in this study were not on prolonged medication of any kind which could have resulted in discrepancy during estimation of TBARS, SOD and GSH level.

To study aims the relationship between breast cancer immunohistopathologic features (ER, PgR and HER2/neu expression, Ki-67 index) and oxidative state (TBA-reactive specimens, reduced glutathione content, glutathione reductase, glutathione transferase, glutathione peroxidase, superoxide dismutase activity, total antioxidant activity), according to patients' menopausal status was analysed.

ER, PgR HER2/neu expression and Ki-67 index in the tumour specimens were examined by immunohistochemistry.

Content of ER and PgR was determined by the method of indirect two-step immunohistochemical study phasic system «Mouse/Rabbit Poly Vue HRP/DAB» (mouse monoclonal antibody (dilution 1: 100 v/v) to estrogen receptors and rabbit polyclonal antibodies to progesterone receptors (dilution 1:1000 v/v) were used, counting immunopositive cells was carried out in areas with the highest manifestation of diaminobenzydyn on 200–300 tumour cells on microscope «Axioskop» (OPTON, Germany), expression of antibodies to the ER and PGR was evaluated positively with intense dark brown nuclei) [28].

Evaluation of protein expression HER2/neu as epidermal growth factor. To the primary polyclonal antibody by Rabbit type HER2/neu at a concentration of 1 : 1200 was attributed. In assessing the level of expression of HER2/neu varying degrees of coloring was taken into account and classified from 0 to 3+ [29].

Estimation the expression of receptors for Ki-67. Proliferation index Ki-67 was calculated as the average of the number of labeled nuclei accounted for 100 nuclei (500–1000 taking into account tumor cells). If the tissue sample was attended by several histology (section proliferative endometrium and simple hyperplasia), these sites were studied separately [28].

TBA-reactive species (TBARS). For the assessment of lipid peroxidation in breast tissues a reaction of lipids with TBA was used [30]. At determination the total antioxidant activity (TAOA) reaction of yolk lipoproteins with TBA was used [31]. Level of reduced glutathione (GSH) was gauged by Elman' method [32]. Superoxide dismutase (SOD) activity was assessed using the assay based on a competition of SOD and nitro blue tetrazolium (NBT) in the reaction medium in a reaction of NADH with phenazine methosulfate in the presence of oxygen [33]. Glutathione reductase (GR) activity was determined by the decrease in NADPH content [33]**.** Glutathione transferase (GST) activity was assessed by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione [34]. Glutathione peroxidase (GP) activity was assessed by method based on the development of a colour reaction of interaction SH-group with the Elman' reagent [35]. Protein content was defined with Folin phenol reagent [36].

Results. Molecular classification of breast tumours based only on immunohistochemistry is quite useful on practical clinical grounds. The analysis of the dependence of receptor status from the patients' age allowed obtaining the following data.

For the group aged 40–50 years without the metastasis in regional lymph nodes ER were absent in 40,7 % of the samples, ones were determined within the limits 1–100 in 29,6 % of the cases, 101–200 in 26 %, 201–300 in 3,7 % of the cases.

On PgR 40,7 % of the patients were in the group with negative signal. In 26 % of the cases the force of responses is within 1–100, 18,5 % – 101–200, 14,8 % –201–300 respectively. The level of expression HER2/neu with force 0,1+ was observed in most of the patients (81,5 %), power 2+ in 3,7 %, 3+ in 14,8 % of patients with primary tumors of the breast. At determination of Ki-67 group with the signal in the range of 0–30 amounted to 52 % of cancer patients aged 40–50 years. In 48 % of cases the force of response is within 31–60. The signal of strength in range of 61–100 was not observed in the sample (Tabl. 1).

To determine the mechanisms leading proliferative processes depending on the signal strength of receptor the response was evaluated in the breast tissue in the presence of metastases.

For the subgroup IB ER negative status was observed in 23 % of the samples, the signal within 1–100 in 38,5 %, 101–200 in 30,8 %, 201–300 in 7,7 % of the biopsies. PgR were absent in 23 % of immunohistochemical samples. Level of PgR was determined in 38,5 % of the cases in both the range 1–100 and 101–200. The samples with content PgR as 201–300 were not fixed. Expression with power HER2/neu 0,1+ was observed in 84,6 % of examined patients, 2+ in 15,4 % of the patients. Women with breast oncopathology in which force responsible for HER2/neu was 2+ were not included to the analysed cohort of patients. For Ki-67 levels in the subgroup IB that had metastasized breast cancer the signal in the range 0–30 was noted in 61,5 % of women, within the 31–60 signal was observed in 38,5 % of cases. Responses in the range 61–100 were not observed.

The analysis of the receptor response in the group consisted of patients older than 50 years revealed the following relationship. The negative response on ER was detected in 27,5 % of the samples. The answer in the range of 1–100 was observed in 20 % of biopsies, 101–200 in 40 %, 201–300 in 12,5 % of the investigated tissue samples. There was no answer on PgR containing in 32,5 % of the samples. The signal with strength within 1–100 was found in 30 % of the samples, 101–200 in 27,5 %, 201–300 in 10 % of the biopsies. Power of HER2/neu expression 0, 1+ was in 90 % of examined patients. There was no difference between the likely numbers of the patients whose level of HER2/neu expression had been identified as 2+ (5 %) and 3+ (5 %). At determining of Ki-67 the signal range from 0 to 30 was observed in 65 % of the cases. In the range of 31–60 response was in 30 % of the biopsies of breast cancer women. Within 61–100 signal was observed only in 5 % of the women.

For the patients' subgroup IIB aged over 50 years the following trend was determined. Connection between estrogen and the obligatory receptor was not observed in 37,5 % of cases. Response with intensity 1–100 was fixed in 25 % of cases, 101–200 in 25 %, 201–300 in 12,5 % of investigated biopsies. PgR negative status was fixed in 50 % of the samples. Answer with intensity 1–100 was observed in 12,5 %, 101–200 in 37,5 % of the biopsies. Response in the range 201–300 was absent. HER2/

Table 1. **Receptor status of breast cancer patients according to age, % (*n*)**

The range of concentrations	Women without metastases		Women with metastases	
	premenopausal	postmenopausal	premenopausal	postmenopausal
The content of estrogen receptors in tissue biopsies				
0	40,7 (11)	27,5 (11)	23 (3)	37,5 (3)
1–100	29,6 (8)	20 (8)	38,5 (5)	25 (2)
101–200	26 (7)	40 (16)	30,8 (4)	25 (2)
201–300	3,7 (1)	12,5 (5)	7,7 (1)	12,5 (1)
The content of progesterone receptors in tissue biopsies				
0	40,7 (11)	32,5 (13)	23 (3)	50 (4)
1–100	26 (7)	30 (12)	38,5 (5)	12,5 (1)
101–200	18,5 (5)	27,5 (11)	38,5 (5)	37,5 (3)
201–300	14,8 (4)	10 (4)	0 (0)	0 (0)
The level of epidermal growth factor HER2/neu in tissue biopsies				
0,1+	81,5 (22)	90 (36)	84,6 (11)	75 (6)
2+	3,7 (1)	5 (2)	15,4 (2)	12,5 (1)
3+	14,8 (4)	5 (2)	0 (0)	12,5 (1)
The level of Ki-67 antigen in tissue biopsies				
0–30	52 (14)	65 (26)	61,5 (8)	37,5 (3)
31–60	48 (13)	30 (12)	38,5 (5)	62,5 (5)
61–100	0 (0)	5 (2)	0 (0)	0 (0)

neu was not detected in 75 % of patients. Expression with intensity of 2+ was detected in 12,5 % and 3+ in 12,5 % of the samples. In patients older than 50 years with metastases Ki-67 signal level in the range of 0–30 was in 37,5 % women. Intensity of response within 31–60 was recorded in 62,5 % of cancer patients. The response in the range of 61–100 was not fixed.

Lipid peroxidation (LPO) is a universal biological process that constantly takes place in the membranes of cells. Its pathological intensification leads to disruption of the structure and, consequently, of the functions of biological membranes. Imbalance in LPO system plays an important role among the risk factors for malignant tumours. On our data in the surrounding background breast tissue at premenopausal women from subgroup IA the content of TBARS was higher on 8,5 % than at older groups (Tabl. 2). TBARS accumulation was higher by 14 % in tumour tissue of patients aged 40–50 years that the parameter of subgroup IIA patients. In premenopausal women TBARS content in the tumour tissue was higher than the index of breast tissue surrounding the tumour. In older women tissue samples significant difference of the parameter was not found.

SOD catalyzes the conversion of the highly reactive and unstable superoxide anion to less reactive and more stable hydrogen peroxide, a critical step in ROS detoxification. In the study of SOD activity as an antioxidant enzyme in the tumour in comparison with surrounding background breast tissues decrease by 25 % was noted at the women under 50. There was a reduction of the enzyme synthesis in the tumour by 27 % at the older patients. When comparing the index in the corresponding tissue samples between the age groups there was no significant difference.

Glutathione peroxidase (GPX) by GSH causes hydrogen peroxide and lipid hydroperoxides to be reduced to water and alcohol related. During this process GSH is oxidized to GSSG with a disulfide bond, which eventually becomes revitalized by a glutathione reductase enzyme. In younger women' breast tissue samples glutathione reductase activity was average by 11 % higher than ones in biopsy of older women. The enzyme activity tended to decrease in tumour tissue in premenopausal women. At the same time in postmenopausal women the difference of these indicators was absent. Similar changes were noted in determining the level of reduced glutathione.

Activity of glutathione transferase and glutathione peroxidase was reduced in the tumour in premenopausal women by 16 and 9 % and in postmenopausal women by 12 and 9 %, respectively. GST and GP activity in the experimental samples had not significant differences depending on the age of the patients.

In determining the TAOA in younger women this index was reduced by 23 % in the tumour in comparison with the surrounding background

Table 2. **Oxidant state of the different age patients without metastasis**

Indexes	Subgroup IA (premenopausal women)		Subgroup IIA (postmenopausal women)	
	surrounding background breast tissue (n = 27)	breast tumour tissue (n = 27)	surrounding background breast tissue (n = 40)	breast tumour tissue (n = 40)
TBARS, nmol/g protein	7,93 ± 0,39	8,45 ± 0,42 *	7,31 ± 0,36 **	7,4 ± 0,37 **
TAOA, conv. Unit	21,75 ± 1,09	16,66 ± 0,83 *	21,13 ± 1,06	14,95 ± 0,75 *, **
GSH, mmol/g protein	16,61 ± 0,83	15,42 ± 0,77 *	15,52 ± 0,78	14,73 ± 0,74
GR, μmol/min × g protein	17,46 ± 0,87	16,73 ± 0,83	15,04 ± 0,75 **	15,07 ± 0,75 **
GST μmol/min × g protein	16,94 ± 0,84	14,25 ± 0,76 *	15,87 ± 0,81	14,42 ± 0,72 *
GP, μmol/min × g protein	18,32 ± 0,92	16,11 ± 0,80 *	16,92 ± 0,85	15,42 ± 0,77 *
SOD, opt. unit/min × g protein	266,28 ± 13,31	200,34 ± 10,02 *	253,63 ± 12,65	185,13 ± 9,25 *

* Significant differences between the indexes of tumour and unchanged tissue, $p \leq 0,05$. ** Significant differences between the relevant indexes depending on the age of the patients, $p \leq 0,05$.

breast tissue. In postmenopausal women TAOA decrease was by 29 % in the tumour tissue. In 40–50-year-old women breast unchanged tissue TAOA did not differ from that in the aged women. However, in younger women cancerous tissue the index exceeded by 11 % than in the older women.

The study of samples of breast tumours metastasized to regional lymph nodes showed the following.

TBARS level had no significant differences between the breast tumour and surrounding background breast tissue of the patients from both subgroups IB and IIB. At the same time, the TBARS accumulation was higher on average by 17 % in the tissues of premenopausal women compared to age women (Tabl. 3).

SOD activity in the surrounding tissue was higher by 42 % than in the tumours of women from subgroups IB. For women from subgroup IIB the corresponding increase amounted to 34 %. In the younger women the enzyme activation exceeded that in the older women biopsies.

In premenopausal women tumours reductive glutathione content showed a tendency to increase in relation to the unchanged tissue. In the women from subgroup IIB such increase was significant.

Depletion of SOD leads to increased accumulation of superoxide in mammary epithelial cells, and enhance apoptosis. Therefore, in response to matrix detachment, cells activate two parallel programs: 1) attenuation of oxidative metabolism of glucose to decrease mitochondrial ROS production and 2) enhancement of antioxidant capacity to detoxify ROS, which allow them to tolerate the lower oxidative stress and prolong survival in suspension. SOD higher expression is associated with advanced tumor grades and breast cancer metastasis, supporting its role in apoptosis resistance and tumor progression [37]. In the surrounding tumour tissue GR activity exceeded the index tumours by 12 % in the younger women. In the elderly patients a significant difference between the compared activities has not been found. The indicator differences depended on the patients' age.

In the patients of subgroup IB GP activity of unchanged tissue was 30 % higher than that in the tumours. In contrast, in the women of subgroup IIB enzyme activity was higher in the tumours. Significant increase in GP activity, the first step of enzyme defense against H_2O_2 and other hydroperoxides, can result from an increased expression of genomic DNA.

In the premenopausal women tumours the glutathione transferase activity had a tendency to decrease. There was a significant activation (average 22 %) of the enzyme in the postmenopausal women.

TAOA was reduced in the tumours of the younger women and the older patients by 30 and 35 %, respectively.

Discussion. We summarize the results of our research on the role of molecule profile and oxidative state biochemical indexes of mediated extranuclear actions in breast tumorigenesis and metastasis.

Table 3. **Oxidant state of the different age patients with metastasis**

Indexes	Subgroup IB (premenopausal women)		Subgroup IIB (postmenopausal women)	
	surrounding background breast tissue (n = 13)	breast tumour tissue (n = 13)	surrounding background breast tissue (n = 8)	breast tumour tissue (n = 8)
TBARS, nmol/g protein	8,94 ± 0,25	8,66 ± 0,34*	7,79 ± 0,30**	7,33 ± 0,12**
TAOA, conv. Unit	24,15 ± 1,31	16,96 ± 1,26*	23,17 ± 1,64	15,03 ± 0,61*, **
GSH, mmol/g protein	18,84 ± 0,95	19,57 ± 1,24*	16,21 ± 0,74	18,74 ± 1,12
GR, μmol/min × g protein	20,69 ± 1,43	18,54 ± 1,14	16,84 ± 1,17**	17,14 ± 0,57**
GST μmol/min × g protein	17,58 ± 1,06	16,86 + 0,511	17,14 ± 0,64	21,06 ± 1,441
GP, μmol/min × g protein	24,83 ± 1,15	19,15 + 0,841	18,51 ± 0,74	19,96 ± 1,011
SOD, opt. unit/min × g protein	365,28 ± 20,41	257,15 + 8,141	294,56 ± 18,09	219,13 ± 11,171

* Significant differences between the indexes of tumour and unchanged tissue, $p \leq 0,05$. ** Significant differences between the relevant indexes depending on the age of the patients, $p \leq 0,05$.

The estrogen receptor (ER) is implicated in the progression of breast cancer. Emerging evidence suggests in addition to exerting its well-studied nuclear functions, ER also participates in extranuclear signalling that involve growth factor signalling components, adaptor molecules and the stimulation of cytosolic kinases. ER extranuclear pathways have the potential to activate gene transcription, modulate cytoskeleton, and promote tumour cell proliferation, survival, and metastasis. Cytoplasmic/membrane ER is detected in a subset of breast tumours, and expression of extranuclear components of ER is deregulated in tumours. The extranuclear actions of ER are emerging as important targets for tumorigenic and metastatic control. Hormonal therapies which block ER functions or local and systemic estrogen production are currently used to treat ER positive breast cancer.

Changes in the receptor profile between primary tumour and metastatic cancers of the breast tissue were marked.

The level of estrogen and progesterone receptors positively correlated with the age of patients with primary tumours. In primary tumours ER positive state was significantly higher than in metastatic specimens of the younger patients. According to our data it can be argued that in the older women the relationship of estrogen with the corresponding receptor (ER+) is observed more frequently (approximately 73 % (on the literature references 75 % [38]) of breast primary tumours had positive estrogen receptor status) than in the women aged 40–50 years (59 % of cases). Inhibition of ER extranuclear actions has the potential to prevent breast tumour progression and may be useful in preventing ER positive metastasis [39, 40].

PgR reduction was determined more often than ER decline at the women aged over 50. Relationship between progesterone and its obligate receptor (PgR+) was found in 67,5 % of the postmenopausal patients and 59 % of the premenopausal women with breast cancer. ER−/PgR-phenotype of the tumour is an important prognostic factor in high risk of distant metastases [41]. In the presence of metastases ER−/PgR − negative status was identified in 23 % of the younger women. The answer by ER was absent in 37,5 % PgR 50 % of the older women: the phenotype of tumours ER+/PgR− was defined more often in the older patients and coincided with the data of the references [42].

Hormonal therapy shows beneficial effects, however, initial or acquired resistance to endocrine therapies frequently occurs, and tumours recur as metastasis. HER2/neu marker can be considered as a factor of unfavourable prognosis, and its high expression is an indicator of high metastatic ability of the tumour and its possible resistance to anti-hormonal therapy. Hormone receptors and the epidermal growth factor HER-2 expression are important pharmaceutical targets for the treatment of patients with metastatic breast cancer. Hormone and HER2/neu receptors discoordination can occur in two directions, but in most cases the alteration was manifested by positivity receptor status reducing [43].

Based on these data the expression of epidermal growth factor (HER2/neu+) was identified in 18,5 % of patients aged 40–50 years and 10 % of patients older than 50 years without regional lymph node metastases. Metastasis is accompanied by increased levels of HER2/neu+ in postmenopausal women (2 % of the samples) and some lower (15,4 % of the biopsies) in premenopausal women.

Amplification/overexpression of HER2/neu directly correlated with premenopausal status and the level of estrogen negative breast cancer phenotype. Women with receptor status of ER+/PgR− and overexpression of HER2/neu are at high risk of tumour recurrence and the tumour characteristics are more aggressive. Signalling through the epidermal growth factor receptor leads to reduce of progesterone expression [44, 45]. This phenomenon is typical for premenopausal patients.

Menopausal status played a very important role in determining HER2/neu and PgR status in ER+ breast cancer patients. HER2/neu was independently inversely associated with PgR only in the postmenopausal women with ER+ breast cancers but not in the premenopausal ones. For this patients with tumour phenotype ER+/PgR+ and HER2/neu a reduction in the risk for local recurrence of the tumour are characterized. ER+, HER2/neu-group of patients with negative PgR receptors seem to be high risk of recurrence and deserve further consideration and the phenomenon is inherent in older patients.

At postmenopausal patients with metastatic tumours phenotypes of triple receptor-negative status (ER−/PgR− and HER2/neu) and ER−/PgR− and HER2/neu+ were determined more often. Such

tumour compared with ER+/PgR+ and HER2/neu subtypes is particularly aggressive [41]. 40–50 year women had no significant difference between positive HER2/neu level for the primary and metastatic tumours.

Ki-67 antibody index is a reliable prognostic marker in determining the number of tumour cells that are in the S period of the cell cycle. Antigen Ki-67 is as an independent prognostic factor with regard to recurrence in patients. At premenopausal women antigen Ki-67 concentration increases indicating the likelihood of recurrence in patients. In the presence of metastases a direct correlation of Ki-67 with patients' age was observed, which was a sign of unfavorable prognosis for this cohort. At the same time Ki-67 expression it is not so dangerous as metabolism activity decreases in the elderly woman. Much more dangerous is the phenomenon when Ki-67 antigen expression directly correlates with overexpression of prooncogene of proliferative growth and low levels of estrogen and progesterone receptors. As a result of our research, this phenomenon is characteristic in premenopausal women with primary tumour and postmenopausal women with metastatic tumours. In the case of such a combination hyper resistance to treatment is determined.

However endocrine resistant breast cancer cells behave very differently at using standard adjuvant therapy and the biology driving this aggressive disease has yet to be defined.

Analysis of the results verified different oxidative stress status depending on patient' age breast cancer arising.

Oxidative stress (due to ROS over-production) leads to genetic instability in the cancer cells (DNA damage and aneuploidy resulting in an increased mutation rate and tumour evolution) via a «bystander effect», resulting in aerobic glycolysis, with lactate and ketone production (the «Reverse Warburg Effect»). Energy-rich metabolites (lactate, pyruvate, ketones, and glutamine) are then transferred to «hungry» cancer cells, promoting mitochondrial biogenesis and anabolic growth in these tumor cells. This event, in turn, promotes tumor growth and protects these cancer cells against apoptosis [46].

The accumulation of TBARS was higher in breast biopsy (as unchanged tissue well as tumour) younger women, resulting from greater intensity of biochemical processes in the body. In addition, TBARS level was significantly higher in tumour tissue than their accumulation in the adjacent tissue cancer. This phenomenon, on the one hand is predictive of high tumour invasiveness as known role of free radicals in biological membrane destabilization. On the other hand, reactive oxygen species formation is a response to stress exposure with further activation of protective systems. Usually in the case of moderately high levels of reactive oxygen species occurs the antioxidant enzyme system activation.

The results of our study suggest that free radical activity is enhanced in cancer breast patients while the antioxidant defence mechanisms are weakened. Enhanced lipid peroxidation accompanied by exhaustion in enzymatic and nonenzymatic antioxidants was observed in breast tumour tissues compared to the corresponding uninvolved adjacent tissues irrespective of menopausal status of the patients.

On the other hand ROS may activate apoptotic pathway as a barrier to tumor metastasis through lipid peroxidation.

Disruption of the delicate balance between free radicals and antioxidants may cause cellular damage and trigger carcinogenesis. A correlation between tissue redox status and tumour progression suggests that upregulation of antioxidants enables tumour cells to counter oxidative stress, thereby conferring a selective advantage for growth compared to corresponding normal cells. Taking into account ability of antioxidants to inhibit the activation of protooncogenes, its reduction in tumours is a negative sign of the neoplasm stability. In tumour tissues the antioxidant system inhibition can result in the changes in cell environmental conditions as a consequence of development of hypoxic tumour cells characteristic that makes them resistant to the therapeutic treatment. In the women aged over 50 the total antioxidant status of the tumours was lower than in the younger cohort. However, GST involved in the detoxification of electrophilic toxins and carcinogens is increased in most of the metastatic tumours at the postmenopausal women. High concentrations of GST may rapidly detoxify anticancer agents, thereby preventing their cytotoxic action [47] and that makes these tumours resistant to chemotherapy. In our opinion, the characteristics of a senescent organism with regards to oxidant-antioxidant status could be causally related to the slow evolution of tumours in old patients.

At the same time, the magnitude of change in tissue oxidant-antioxidant status was, however, more pronounced in the premenopausal patients compared to the postmenopausal ones. In some tumours the increased activity of antioxidant enzymes and GSH level were observed. Cancer cells with increased activities of antioxidant enzymes are presumed to escape recognition by cytotoxic lymphocytes and it may offer a selective growth advantage to tumour cells over their surrounding normal counterparts [48]. As activation of various antioxidant enzymes (superoxide dismutase, catalase, glutathione-S-transferase and glutathione reductase) and the levels of reduced glutathione are important to protect cancer cells against cytotoxic hydrogen peroxide these neoplasms are characterized by high stability and overexpression HER2/neu with subsequent amplification of proliferative processes. Damage of biological membranes due to imbalance of oxidative processes, in our opinion, affects activation of membrane receptors, including estrogen and progesterone ones, violates of signalling pathways an intercellular interactions. Such high SOD expression evidently correlated with the negative status of estrogen receptor (ER) and progesterone receptor (PgR). It was associated with tumor progression and is a predictor of poor prognosis. High GSH level was associated with the tumors negative for the estrogen receptor (ER) and metastasis.

Conclusion. This data illustrates that breast cancer arising in young women is a unique biologic entity driven by unifying oncogenic signaling pathways characterized by less hormone sensitivity due to low estrogen and progesterone receptor response, significant violation of oxidative status and higher HER2/neu and Ki-67 expression as indicators of proliferative activity which are the triggers of recurrence and metastasis in the patients. The risk of breast cancer increases with age as a result of hormonal profile violation in women organism of the whole and local regional steroid status. Yet in the postmenopausal women the risk of tumour dissemination is lower than in the premenopausal women due to the metabolism changes with age. Frequently they have significant comorbidities and competing health risks. Elderly patients' neoplasms are stable and resistant to the effects of hormonal, chemo- and radiotherapy. The obtained data is recommended to be considered in conducting individual treatment and especially hormone therapy of patients with breast cancer of different ages.

ИММУНОГИСТОХИМИЧЕСКИЙ И БИОХИМИЧЕСКИЙ АНАЛИЗ ОПУХОЛЕЙ МОЛОЧНОЙ ЖЕЛЕЗЫ ПАЦИЕНТОК РАЗЛИЧНОГО ВОЗРАСТА

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Иммуногистохимическое и биохимическое изучение инфильтративной протоковой карциномы молочной железы и ткани, прилегающей к опухоли, выявило особенности молекулярного профиля и характеристик оксидантно-антиоксидантного состояния новообразований в зависимости от возраста пациенток и наличия метастазов в регионарные лимфоузлы. Установлены некоторые причины высокой агрессивности и низкой гормональной чувствительности опухолей у женщин в пременопаузе, а также стабильности и высокого метастатического потенциала новообразований у женщин в постменопаузе.

ІМУНОГІСТОХІМІЧНИЙ ТА БІОХІМІЧНИЙ АНАЛІЗ ПУХЛИН МОЛОЧНОЇ ЗАЛОЗИ ПАЦІЄНТОК РІЗНОГО ВІКУ

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Імуногістохімічне і біохімічне вивчення інфільтративної протокової карциноми молочної залози і тканини, прилеглої до пухлини, виявило особливості молекулярного профілю і характеристик оксидантно-антиоксидантного стану новоутворень в залежності від віку пацієнток та наявності метастазів у регіонарні лімфовузли. Встановлені деякі причини високої агресивності та низької гормональної чутливості пухлин у жінок у пременопаузі, а також стабільності та високого метастатичного потенціалу новоутворень у жінок у постменопаузі.

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