МЕДИЦИНСКИЕ НАУКИ

STATE OF GLUTATHIONE ANTIOXIDANT SYSTEM IN BLOOD LYMPHOCYTES AT OVARIAN CANCER

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Abstract. Activation of lipid peroxidation (LP) processes in blood lymphocytes of women with ovarian cancer was shown. Thus, in the control group the concentration of *malonic dialdehyde* (MDA) in blood lymphocytes was (62.1±4.5) μmol/mg protein. This value increases significantly up to (96.9±7.4) μmol/mg protein at ovarian cancer (OC). A similar situation is observed when determining the concentration of MDA in serum. Thus, in the control group it reaches (7.5±0.6) μmol/L. When OC, the processes of LP are intensified in 1.6 times relative to the control group. Simultaneously, with the intensification of LP processes the corresponding changes in the activity of enzymes of the glutathione antioxidant system were revealed. It was shown that in practically healthy women the concentration of glutathione is (17.8±1.5) nmol/mg protein. At OC, this value increases in 1.4 times and reaches (25.3±2.2) nmol/mg protein. Also a significant difference in glutathione peroxidase activities was revealed. In the control group it is (161.8±12.7) nmol GSH/min·mg protein. At OC this value decreases in 1.7 times up to (96.1±8.8) nmol GSH/min·mg of protein. Regarding the activity of glutathione reductase, in the lymphocytes of the control group it is equal to (51.9±5.1) nmol NADPH/min·mg protein. At OC, this activity is reduced to (35.1±3.1) nmol NADPH/min·mg protein, that is in 1.4 times lower than in control group. The glutathione transferases (GSTs) activity of the control group is (114.9±9.2) nmol GSH/min·mg protein. With the development of OC (stage III-IV) the activity of GSTs increases to (138.8±11.6) nmol GSH/min·mg of protein, that is in 1.2 times more than in control, however these changes are not statistically significant. Thus, OC significantly intensifies LP processes in both lymphocytes and blood plasma. Simultaneously, the development of OC significantly reduces the activity of two major antioxidant enzymes - glutathione peroxidase and glutathione reductase.

Key words: ovarian cancer, lymphocytes, *malonic dialdehyde*, glutathione, glutathione peroxidase, glutathione reductase, glutathione-S transpherase.

Introduction. According to modern ideas, the development of pathological processes in the organism is accompanied by a violation of the antioxidant protection mechanisms of cells [1-3]. During tumor growth, oxidative free radical processes are initiated in particular in phospholipids of cell membranes containing polyunsaturated fatty acids. Intensification of lipid peroxidation leads to the accumulation of toxic products which leads to DNA damage, gene mutations and reducing the body's resistance which are considered the initial stages of carcinogenesis [2, 4, 7, 12]. However, the buffer capacity of the antioxidant system under normal conditions is quite large and is provided by various components. An important role among the antioxidant system of the cell is occupied by the glutathione system which consists of both enzymatic (glutathione peroxidase, glutathione reductase, glutathione S-transferase) and non-enzymatic (reduced glutathione) components [5, 9-11]. Although the glutathione system has been the the subject of many studies there is no consensus in the literature regarding to its role in malignant growth. Because intracellular lymphocyte metabolism is based on the physiological and biochemical ability of these cells to respond rapidly to any changes in homeostasis of the organism the modulation of enzyme activity in lymphocytes occurs much earlier than their morphological and functional parameters change.

This allows to use the status of lymphocytes as a "metabolic mirror" of the organism. We suggest that peripheral blood lymphocytes may be a convenient and adequate model for studying the peculiarities of the functioning of regulatory systems in both healthy individuals and at neoplastic ovarian transformation. Therefore, analysis of the functional state of the glutathione system in blood lymphocytes at ovarian

cancer will reveal its role in the pathogenesis and prognosis of the disease.

Material and methods. The study was performed on blood lymphocytes of practically healthy women and patients with neoplastic ovarian changes. The total number of practically (clinically) healthy women representative by age (mean age 53.8±5.4 years) was 40 people. This group was formed by volunteers from the staff of Danylo Halytsky Lviv National Medical University and employees of the Lviv State Regional oncology treatment and diagnostic Center.

The group of women with ovarian cancer (stage III-IV) consisted of 78 women aged 24-75 years (mean age 55.4±5.3 years) who were hospitalized at the Lviv State Regional oncology treatment and diagnostic Center in the period 2016-2019 and underwent a full clinical and laboratory examination. The study included patients diagnosed with ovarian cancer without comorbidities at the start of the research.

Clinical diagnoses were established on the basis of a wide range of general clinical, laboratory, instrumental and special oncological research methods. In addition, the level of tumor marker glycoprotein CA-125 in serum was determined to differentiate almost healthy women and diagnose ovarian cancer [8]. All ovarian cancer patients and practically healthy persons were informed in detail about the purpose, objectives and terms of research and gave written informed consent to participate in research on blood samples. Any violations of moral and ethical norms were not revealed by the Bioethics Commission of Danylo Halytsky Lviv National Medical University.

Peripheral blood lymphocytes were isolated by the method of A. Boyum (1968). Blood diluted 1:1 with physiological saline was layered in a density gradient of phycol-triumbrast (ρ=1.08 g/cm^3) and centrifuged for 20 minutes at 500 g. Removed interphase rings of mononuclear cells were washed twice for 10 minutes with physiological saline. After the last centrifugation a small amount of physiological saline was added to the pellet, resuspended, and the number of living and dead cells was counted in the Goryaev chamber using trypan blue. The integrity and viability of blood lymphocytes in all experiments was at least 95%. Saponin was added to the suspension to permeabilize blood lymphocyte membranes and reveal latent enzymatic activities [6].

Plasma lipid peroxidation was assessed by the concentration of malonic dialdehyde (MDA) in the reaction with 2-thiobarbituric acid (Timirbulatov, 1981). Glutathione peroxidase (GP) activity was determined by the rate of GSH oxidation in the presence of tert-Butyl hydroperoxide (Moin, 1986).

The determination of glutathione reductase (GR) activity was performed by decreasing the NADPH content (Carlberg, 1985). Glutathione S-transferase (GsT) activity was determined by the rate of enzymatic formation of glutathione S-conjugate in the GSH reduction reaction with 1-chloro-2,4 dinitrobenzene (Karpishenko, 2002). The content of reduced glutathione (GSH) was calculated as the difference between the concentrations of total and oxidized glutathione using Elman's reagent (Anderson, 1985).

The significance of changes between the statistical characteristics of two alternative data sets was established by Student's t-test. The difference in probability value $p<0.05$ was considered significant.

Results and discussion. We conducted a comparative study of lipid peroxidation (LP) and glutathione system in practically healthy women and women with ovarian cancer (OC). Activation of LP processes in blood lymphocytes of women with ovarian cancer was shown by determining the concentration of malonic dialdehyde (MDA) which is a secondary product of lipid peroxidation. Thus, in the control group the concentration of MDA in blood lymphocytes in calcium-free medium was (62.1±4.5) μmol/mg protein (Fig. 1). At OC this value increases statistically and significantly up to (96.9±7.4) μmol/mg protein ($p<0.001$).

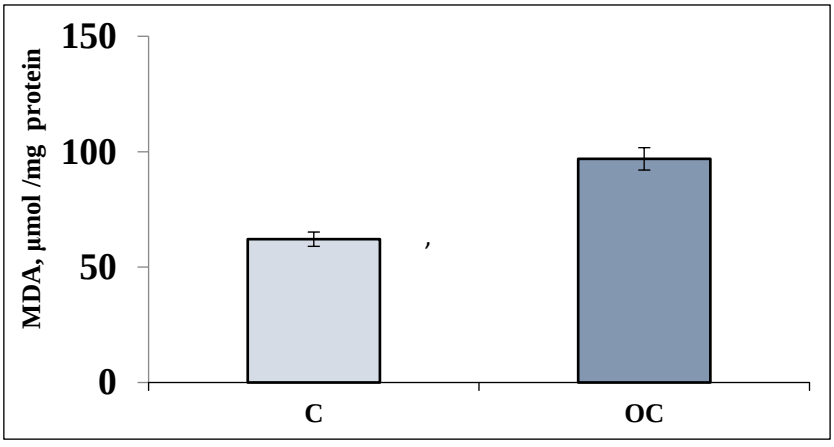

Fig. 1. The concentration of malonic dialdehyde in the lymphocytes of practically healthy women (C) and women with ovarian cancer (OC)
*Comment: *p<0,001 compared to practically healthy women*

It should be noted that the intensity of lipid peroxidation in lymphocytes depends on the presence of calcium ions in the medium (Fig. 2).

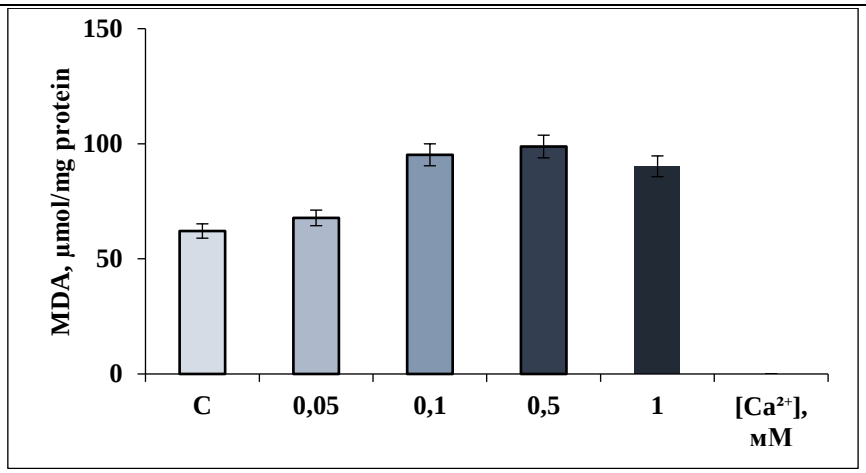

Fig. 2. The dependence of the level of malonic dialdehyde in blood lymphocytes on the concentration of Ca^{2+}.

It can be seen that at the presence 0.5 mM Ca^{2+}in incubation medium lipid peroxidation increases in 1.6 times ($p<0.001$). However, it should be noted that these are not physiological concentrations of Ca^{2+}. They exceed them by two orders of magnitude.

A similar situation as in lymphocytes is observed when determining the concentration of MDA in serum. In the blood serum of the control group it reaches (7.5±0.6) µmol/l (Fig. 3). At OC, LP processes intensify in 1.6 times relative to the control group ($p<0.001$). Thus, at OC LP processes are significantly intensified, in blood plasma in 1.6 times ($p<0.001$) and in blood lymphocytes in 1.6 times ($p<0.001$).

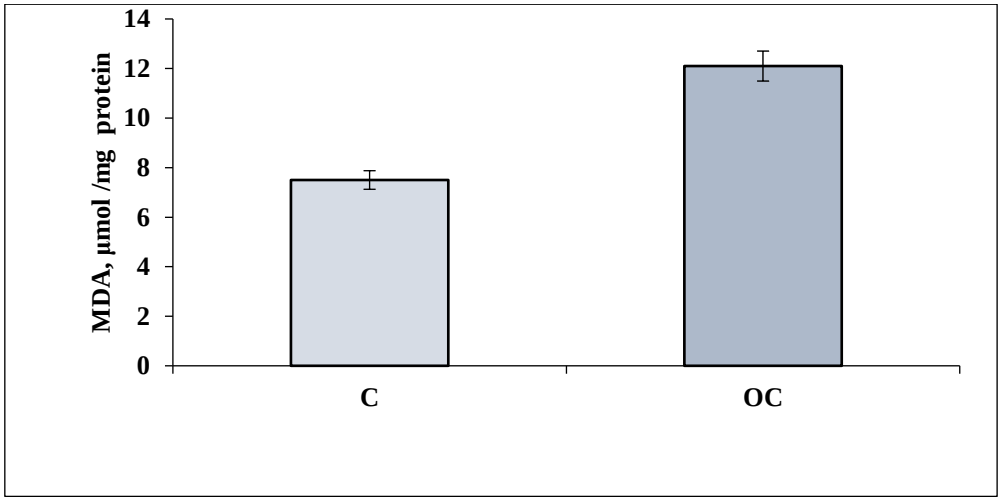

Fig. 3. The concentration of malonic dialdehyde in the serum of practically healthy women (C) and women with ovarian cancer (OC).
*Comment: *p<0.001 compared to practically healthy women*

Simultaneously with the intensification of LP processes the corresponding changes in the activity of enzymes of the glutathione system were revealed (Table 1).

Table 1

The state of the glutathione antioxidant system and lipid peroxidation in practically healthy women and patients with ovarian cancer, M±m, n=18-22.

Groups / Indicators	Practically healthy women	Women with ovarian cancer, stage III-IV
GSH, nmol/mg protein	17.8±1.6	25.3±2.1*
GP, nmol GSH/min·mg of protein	161.8±12.7	96.1±8.8***
GR, nmol NADPH/min·mg of protein	51.9±5.1	35.1±3.1*
GsT, nmol GSH/min·mg of protein	114.9±9.2	138.8±11.6

Comment: $^{*}p<0.05$,$^{***}p<0.001$ compared to practically healthy women

Thus, it is shown that in practically healthy women the concentration of glutathione is (17.8±1.5) nmol/mg protein. At OC, this value increases in 1.4 times and reaches (25.3±2.2) nmol/mg protein ($p<0.01$). Also a significant difference in glutathione peroxidase activities was revealed. In the control group it was (161.8±12.7) nmol GSH/min·mg protein. At OC this

value decreases in 1.7 times up to (96.1±8.8) nmol GSH/min·mg protein ($p<0.001$).

Regarding the activity of glutathione reductase in the lymphocytes of the control group it is equal to (51.9±5.1) nmol NADPH/min·mg protein. At OC enzyme activity is reduced up to (35.1±3.1) nmol NADPH/min·mg protein, that is lower in 1.4 times ($p<0.05$) than in the control group.

The glutathione S-transferases activity of the control group is (114.9±9.2) nmol GSH/min·mg protein. With the development of OC (stage III-IV) GSTs activity increases up to (138.8±11.6) nmol GSH/min·mg protein, that is in 1.2 times more than in the control group, however these changes are not statistically significant ($p>0.05$).

Thus, it is known from the literature that lipids, especially polyunsaturated fatty acids are very sensitive to free radicals that initiate lipid peroxidation [1]. One of the *final products* of LPO is MDA, which due to its high cytotoxic effect, in particular on the antioxidant system, can act as a co-carcinogenic agent [1, 8, 9]. From the data obtained by us it is seen that at OC the concentration of MDA is increased significantly, whereas activities of two main antioxidant enzymes glutathione peroxidase and glutathione reductase are decreased.

Conclusion. 1. At ovarian cancer the processes of lipid peroxidation in lymphocytes and in blood plasma are significantly intensified.

2. With the development of OC in blood lymphocytes, the activities of two main antioxidant enzymes glutathione peroxidase and glutathione reductase are significantly reduced in comparison with healthy donors.

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ПРОФЕССИОНАЛЬНАЯ ПОДГОТОВКА ПРЕПОДАВАТЕЛЕЙ И ИНСТРУКТОРОВ ПРЕДМЕТА «ПЕРВАЯ ПОМОЩЬ» – ВАЖНОЕ ЗВЕНО СИСТЕМЫ БЕЗОПАСНОСТИ

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