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FEATURES OF OXIDATIVE METABOLISM AND GENETIC DISORDERS IN PERIPHERAL BLOOD LYMPHOCYTES OF PATIENTS WITH PRIMARY CERVICAL CANCER

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Background: **The combination of chemo- and radiotherapy used as main treatment of locally advanced cervical cancer (CC) may lead to side effects in healthy cells, which undermine the effectiveness of treatment and quality of life. The assessment of damage level in healthy radiosensitive cells from the tumor environment before the treatment is important in order to predict and prevent remote side effects of radiation.** ***Aim:*** **To study the oxidative metabolism and genetic disorders in peripheral blood lymphocytes (PBL) of primary CC patients in order to evaluate the possibilities of predicting radiation complications based on the molecular and biological properties of PBL.** ***Materials and Methods:*** **Peripheral blood samples were collected from 13 primary CC patients $T_{1-4}N_{0-1}M_{0-1}$, and PBL were routinely isolated. The oxidative metabolism (mitochondrial trans-membrane potential, superoxide anion radical ($O_2^{\bullet-}$) generation, reactive oxygen species (ROS) production in PBL as well as the level of SH-groups in plasma and pro/antioxidant ratio in hemolysates were examined. The development of genetic instability was determined by estimation of DNA double-strand breaks (DNA-DSB), frequency and spectrum of chromosome aberrations and apoptosis.** ***Results:*** **The marked increase in the intensity of $O_2^{\bullet-}$ generation in PBL (1.5-fold), depletion of SH-groups content (1.6-fold) and a shift in the pro-antioxidant balance (1.4-fold) towards its prooxidant component were observed in the blood of primary CC patients as compared to healthy individuals. These oxidative stress related events were accompanied by an increase in the level of DNA-DSB (2.1-fold), apoptosis (3.5-fold) and frequency of cells with chromosome aberrations (3.9-fold). On the contrary, significant decrease in mitochondrial trans-membrane potential (2.0-fold) and ROS generation in PBL (4.0-fold) were detected.** ***Conclusion:*** **Preliminary data indicate a violation of redox processes regulation, a shift in the pro-antioxidant balance towards its pro-oxidant component, accompanied by an increase in the level of DNA damage, development of genetic instability and apoptotic death of blood lymphocytes in primary CC patients.**

Key Words: **cervical cancer, oxidative metabolism, DNA double-strand breaks, chromosome aberration, apoptosis, peripheral blood lymphocytes.**

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Cervical cancer (CC) is the fourth-most common cancer in women by incidence and mortality and presents a global healthcare concern among the female population both in Ukraine and in the world, especially among women of reproductive age. Incidence and mortality vary highly between developed and developing countries and more than 80% of new cases of CC are diagnosed in the latter [1]. According to the International Agency for Research on Cancer, the incidence of CC ranges from 4.4 to 42.7/100,000 women. According to the National Cancer Registry of Ukraine, the age-standardized incidence rate of this disease in 2020 was 16.1/100,000 women. CC ranks third in the incidence of malignant neoplasms and first in mortality in the 18–29 age group and second in morbidity and mortality in the 30–54 age group [2].

One of the leading and most effective approaches in the treatment of locally advanced CC is combined radiation therapy consisting of external beam radiation therapy and brachytherapy [3, 4]. This combination allows to significantly escalate the dose to the tumor and minimize radiation exposure to the surrounding organs and tissues. It has been shown that the addition of brachytherapy to complex treatment regimens improves local control, which is also reflected as an increase in overall survival of patients [3–5]. An additional use of chemotherapy, which serves also as a radiosensitizer, allows further increase in overall survival of patients by an average of 5% [5, 6].

The use of combined radiation and chemotherapy leads to the termination of the hormone-producing function of the ovaries in CC patients of reproductive age. This is due to atrophic processes in the lower parts of the genitourinary system [7], genitourinary syndrome of menopause, the manifestations of which significantly affect the quality of life of patients in the long term after treatment [8, 9].

Combined radiation and chemotherapy may lead to side effects in healthy cells and tissues that are located in the irradiated zone. This negatively affects the effectiveness of treatment and quality of life. Therefore, an important approach in research is the assessment of the level of damage in healthy radiosensitive cells that are in direct contact with the tumor, such as lymphocytes of the circulating blood pool. This is particularly important before the beginning

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Abbreviations used: CC – cervical cancer; DNA-DSB – DNA double-strand breaks; MTP – mitochondrial trans-membrane potential; $O_2^{\bullet-}$ – superoxide anion radical; PAR – pro/antioxidant ratio; PBL – peripheral blood lymphocytes; ROS – reactive oxygen species; SH-groups – sulfhydryl groups.

of concomitant chemo-radiotherapy in order to predict and prevent remote side effects of radiation including the development of secondary cancer. Molecular disorders, chromosomal and other abnormalities in healthy cells of cancer patients change the functional status of these cells including radiosensitivity. Additional radiation-induced damage in these cells due to therapeutic irradiation may contribute to a high risk of distant radiation complications from the healthy tissues, organs surrounding the tumor, including circulating blood pool cells.

Variations in hematological indicators may have prognostic value for treatment of cancer patients. In particular, a decrease in the number of T-lymphocytes and cytotoxic T-lymphocytes in the peripheral blood of CC patients after chemotherapy is a prognostic factor of a favorable treatment outcome [10]. The course of radiation therapy causes a decrease in the percentage of lymphocytes and an increase in the ratio of neutrophils to lymphocytes in some patients, which is associated with a later stage of the disease (larger tumor size and metastasis to the lymph nodes) and low rates of overall survival or survival without progression [11, 12]. A number of studies have shown that changes in mitochondrial trans-membrane potential (MTP) and reactive oxygen species (ROS) production in peripheral blood lymphocytes (PBL) can be used as markers characterizing the development and consequences of treatment of various diseases [13, 14].

The development of many pathological processes is associated with an increase in free radical reactions, which leads to the oxidative damage of various biomolecules. Data from the literature indicate that oxidative stress, as an imbalance between pro- and antioxidants, plays a significant role in the development and progression of CC [15, 16]. At the same time, a violation of the pro-oxidant-antioxidant balance in favor of pro-oxidants leads to excessive formation of free radicals, in particular ROS, the main ones of which are superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). The generation of free radicals, in its turn, leads to DNA damage and genetic instability.

The sulfhydryl groups (SH-groups) of proteins and low molecular weight compounds play an important role in antioxidant processes in cells. They also have a significant role in the numerous biological processes, particularly in apoptosis, proliferation, metabolism and regulation of transcription. The exchange of thiol-disulfide has been found to contribute to protein folding and its stability as well to affect the redox potential of cells and proteins [17]. The active forms of oxygen and nitrogen formed both in normal physiological processes as well as in response to the radiation play a special role preceding formation of genetic instability of cells. Well-known is a correlation between the apoptosis-associated changes in cells and their radiosensitivity. The increased induction of chromosomal aberrations and formation of micronuclei in lymphocytes of cancer patients after irradiation may be explained in many cases by apoptosis as a mechanism of elimination of cells with damaged DNA and maintaining the genetic stability [18].

Therefore, it is important and promising to search for biological predictors of the complications severity in patients with CC after combined chemo-radiotherapy. Our previous study shows that comprehensive examination of gynecological cancer patients with the use of radiosensitive blood cells as radiobiological indicators provides an opportunity to predict the possibility of long-term post-radiation complications and prevent their development [19].

The aim of the study was to assess the features of oxidative metabolism and genetic disorders in PBL of primary CC patients in order to evaluate the possibilities of predicting radiation complications based on the molecular and biological properties of the radiosensitive blood cells.

MATERIALS AND METHODS

In the study, peripheral blood was collected from primary CC patients before the treatment. 13 CC patients 30 to 66 years old (mean age 50.0 years) were diagnosed and treated at the National Cancer Institute of the Ministry of Health of Ukraine (Kyiv) in 2022. Blood samples of 21 conditionally healthy women (control) aged from 21 to 69 years (mean age 42.4 years) served as control. Most patients had squamous cell carcinoma, while one patient had endocervical adenocarcinoma and one — low-differentiated squamous cell carcinoma. All patients were informed about the examination and provided a written informed consent to participate in the study, which was approved by the Ethics Committee of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine. The clinical diagnosis of CC in all patients was confirmed histologically and the stage of the tumor process was determined according to the International Classification of Tumors (TNM, 8th edition, 2017). All patients enrolled in the study did not previously receive chemo- or radiotherapy. The clinical and morphological characteristics of the examined CC patients are provided in Table 1.

Table 1. Clinical and morphological data of CC patients

Indicators		Average age, years	n	%
Overall		50.0	13	100
Stage of the disease, TNM	$T_1N_0M_0$	51.5	4	30.7
	$T_1N_1M_0$	65.0	1	7.7
	$T_2N_0M_0$	60.0	3	23.1
	$T_2N_1M_0$	38.7	3	23.1
	$T_3N_0M_0$	53.0	1	7.7
	$T_4N_1M_1$	30.0	1	7.7
Differentiation grade, G	G_2	52.7	7	53.8
	G_3	46.8	6	46.2

Peripheral blood was sampled in the standard sterile 6 ml Vacutainer tubes with Li-heparin anticoagulant (F.L. Medical, Italy). PBL were isolated by centrifugation using Histopaque-1077 (Sigma-Aldrich, USA) according to the manufacturer's instructions [20]. Blood plasma was obtained by centrifugation (15 min

at 1200 g; 20 °C) and stored at 4 °C up to 1 h. The number of viable cells was counted with trypan blue (Sigma, USA) supravital staining [21] and the concentration of lymphocytes was adjusted to 1.0 million per ml.

MTP in PBL was determined using JC-1 dye [22, 23] with some modifications [24]. Fluorescence measurements (λex = 485 nm, λem = 528 nm and λex = 485 nm, λem = 590 nm) were performed using a Sinergy HT plate reader (USA). The wells contained 100 µl of lymphocyte suspension (50,000 cells) stained with JC-1 (20–25 min) and twice washed with PBS. Control wells contained 100 µl of intact cells. The level of MTP was calculated as the ratio of the intensity of red (λem = 590 nm) and green (λem = 528 nm) fluorescence of cells (590 nm/528 nm).

The intensity of $O_2^{\cdot-}$ generation by PBL was evaluated by the lucigenin chemiluminescence method [25]. Measurements were carried out on the AutoLumat LB 953 device (Germany) during 72 s (puls/72 s) [26].

The intensities of the ROS production in PBL were determined using the fluorescent dye 2,7-dichlorofluorescin diacetate (DCFH DA) [27, 28] with some modifications [24]. Fluorescence measurements (λex = 485 nm, λem = 528 nm) were performed on a Synergy HT plate reader. The results were calculated according to the calibration curve and expressed as mM hydrogen peroxide per 1000 cells per h (mM/10^3 cells/h).

The balance of free radical processes in blood was evaluated by determining the pro/antioxidant ratio (PAR) in hemolysates by the method of H_2O_2-induced chemiluminescence on the AutoLumat LB 953 [26, 29]. The total amount of light emitted in 3 min of the reaction (puls·10^3/180 s) was registered reflecting the PAR of the sample.

The level of DNA double-strand breaks (DNA-DSB) in PBL was determined by the method of electrophoresis of individual cells (Comet assay) under neutral conditions [30, 31]. The percentage of DNA in the "tail" of the comet was used as an indicator of DNA damage [32].

The metaphase analysis of PBL chromosome aberrations was performed in the first mitosis (at 48 h of cell cultivation) according to the standard protocol [33]. Phytohemagglutinin (Gibco, USA) was used as a T-lymphocyte mitogen. For each patient, 200–300 metaphases were analyzed.

The content of apoptotic cells in PBL samples was assessed by flow cytometry (DxFlex, Beckman Coulter Biotechnology Co. Ltd with Kaluza C software for clinical analysis) and expressed as percentage of hypodiploid cells. Preliminary processing of the cells includes ribonuclease and propidium iodide staining according to the technique described in [34].

Evaluation of the content of SH-groups of proteins and peptides in blood plasma was performed by spectrophotometric method in modification [35].

Statistical analysis. The values were expressed as the mean ± standard error of the mean. Student's *t*-test was used to evaluate the significance of the differences between individual groups. The difference was considered significant at $p \leqslant 0.05$.

RESULTS AND DISCUSSION

The development of many pathological processes is associated with an increase in free radical formation, which leads to oxidative stress development. This process is accompanied by excessive formation of ROS, largely $O_2^{\cdot-}$ which can transform into other free radical forms.

The intensity of $O_2^{\cdot-}$ generation in PBL of CC patients was increased as compared to healthy individuals (Table 2). The average value of $O_2^{\cdot-}$ generation in PBL of the patients was 1.55 times ($p \leqslant 0.05$) higher than in the control group. The analysis of the PAR also revealed a significant increase in its level in the hemolysate of patients by 1.37 times, compared to healthy individuals, which indicates the enhancement of prooxidant processes in the blood of CC patients.

Table 2. Pro- and antioxidant processes in blood of primary CC patients

Indicators	Patients with CC, M ± m	Healthy individuals, M ± m
$O_2^{\cdot-}$, puls/72s	1186.36 ± 94.08*	767.20 ± 39.80
(min–max)	(742.67–3109.00)	(488–1390)
PAR, puls·10^3/180s	24.48 ± 2.09*	17.34 ± 0.73
(min–max)	(9.55–56.10)	(6.92–31.11)
MTP, 590 nm/528 nm	8.051 ± 1.760*	16.674 ± 3.680
(min–max)	(2.658–26.634)	(3.324–45.636)
ROS, mM/10^3 cells/h	6.010 ± 0.493*	24.119 ± 3.611
(min–max)	(2.507–9.770)	(5.319–64.307)
SH-groups, mM	0.31 ± 0.03*	0.50 ± 0.01
(min–max)	(0.23–0.54)	(0.33–0.74)

Notes: *significant difference compared to healthy individuals, $p \leqslant 0.05$.

A significant decrease in MTP (by 2.0 times) was observed in the PBL of CC patients compared to healthy individuals. We observed the significant individual variability of the MTP level for both CC patients and the healthy individuals of the control group (Table 2). The coefficients of variation for values of experimental groups were 0.79 and 0.83 respectively.

The intensity of ROS production in the PBL of CC patients significantly outweighed the changes in the MTP level (Table 2), when we observed significant ROS level reduction by 4.0 times in PBL of CC patients as compared to the control group. However, the range of individual data variation for this indicator in the group of CC patients was significantly smaller (coefficient of variation = 0.30) in comparison with healthy individuals (coefficient of variation = 0.69).

The intensification of $O_2^{\cdot-}$ generation by PBL of CC patients and significant increase of PAR level were accompanied by the depletion of SH-groups content in the blood plasma of CC patients. The average level of SH-groups was reduced by 38% in blood of CC patients as compared to the group of healthy individuals (Table 2). At the same time, the level of spontaneous apoptosis in PBL of CC patients was elevated and exceeded 3.5 times its value in the control group (Table 3).

Thus, preliminary data were obtained that indicate a decrease in the content of SH-groups and an increase in the level of spontaneous apoptosis in the blood of CC patients as compared to their values

in conditionally healthy donors. This suggests a violation in regulation of redox processes under oxidative stress conditions, which is characteristic of the presence of a pathological process and may indicate a decrease in the antioxidant properties of the blood.

Obtained results regarding decreased MTP level coincided with relatively low intensity of ROS formation by PBL in CC patients were rather unexpected. It is known that mitochondrial dysfunction is usually associated with a low level of MTP caused by increased ROS production. Its persistent decrease may indicate disorder of the electron transport chain [36]. A reduced level of MTP and an increased intensity of ROS formation in the PBL are observed in some diseases [37, 38] and upon the use of cytotoxic substances [39, 40]. On the contrary, the effect of substances with protective properties can be accompanied by an increase in MTP and a decrease in ROS production [41].

However, there are studies that show unidirectional changes in TMP and the intensity of ROS production [42, 43], and that destruction of MTP may not be related to the excessive formation of ROS [44]. It can be assumed that a significant decrease in the MTP and low production of ROS in the PBL of examined CC patients may be associated with redistribution in the formation of free radical forms. Yet, we have seen an increased formation (1.5 times) of comparably more destructive ROS such as $O_2^{\cdot-}$ by the PBL of these patients (Table 2).

Obtained data suggest the activation of free radical oxidation processes in PBL of CC patients perhaps due to the influence of tumor metabolism and the development of oxidative stress. This is confirmed by other researchers [45, 46], particularly Zahra *et al*. [16] showed an increase in the lipid peroxidation in the blood of patients with CC compared to healthy individuals, which becomes more pronounced in late stages of the tumor process. Even more significant increase in oxidative processes was observed in metastasizing CC [47].

Besides, free radicals attack other biomolecules, including nucleic acids. Studies have shown that under the influence of ROS, about 10,000 DNA base changes occur at the level of one cell per day [48]. ROS cause changes in purine and pyrimidine bases, loss of purines, as well as single- and DNA-DSB and cross-links [49].

We found out a significant increase in the number of DNA-DSB (by 2.12 times) in PBL of CC patients as compared to healthy individuals (Table 3). DNA-DSB are mostly responsible for chromosomal aberrations formation, which in its turn can trigger apoptosis or neoplastic transformation of cells.

Table 3. DNA damage and apoptosis in PBL of primary CC patients

Indicators	Patients with CC, M ± m	Healthy individuals, M ± m
DNA-DSB,%	8.65 ± 1.11*	4.08 ± 0.65
(min–max)	(3.90–14.87)	(1.66–6.47)
Apoptosis, %	6.90 ± 0.43*	1.97 ± 0.16
(min–max)	(2.15–8.63)	(1.24–4.53)

Notes:*significant difference relative to healthy individuals, $p \leqslant 0.05$.

Thus, characteristic features of oxidative metabolism were revealed in the blood of CC patients before the start of antitumor therapy that consist in marked increase in the intensity of $O_2^{\cdot-}$ generation in PBL, decrease in the content of SH-groups and a shift in the pro-antioxidant balance in the blood towards its pro-oxidant component. These oxidative stress related events are accompanied by an increase in the level of DNA-DSB damage, apoptosis and point to the development of genome instability. Such changes in the blood of patients with CC can provoke the occurrence of complications in the early and long terms after a course of combined radio and chemotherapy.

Our previous experience, accumulated by examining oncogynecologycal patients with relevant biological indicators, suggests the necessity to study mutational changes in the genome of highly radiosensitive somatic cells of patients prior to anticancer therapy [50, 19]. In order to assess genome instability in T-lymphocytes of patients with CC, studies have been carried out to estimate the background frequency and spontaneous chromosome aberration spectrum before the beginning of combined chemoradiotherapy. The average frequency of cells with chromosome aberrations in blood lymphocytes of the examined patients was 7.08 ± 0.84% and exceeded almost 4.4 times the value of this index in the control group and more than twice the upper limit of the average population level. The total frequency of chromosome aberrations in PBL of CC patients comprised 7.84 ± 0.91/100 analyzed cells, i.e. 1.12 aberrations per aberrant cell, which exceeded the value of this index in the control group (Table 4).

Table 4. Results of cytogenetic screening of PBL in primary CC patients

Cytogenetic indicators	Patients with CC,% per 100 cells, M ± m	Healthy individuals, M ± m
Frequency of cells with chromosome aberrations	7.08 ± 0.84*	1.81 ± 0.24
Total frequency of chromosome aberrations	7.84 ± 0.91*	1.81 ± 0.24
Frequency of chromatid type aberrations	5.22 ± 0.60*	1.14 ± 0.24
Frequency of chromosomal type aberrations	2.61 ± 0.20*	0.67 ± 0.24
Dicentrics	0.16 ± 0.10	0
Fragments	1.01 ± 0.10	0.67 ± 0.24

Notes:*significant difference relative to healthy individuals, $p \leqslant 0.05$.

The ratio of the chromatid to chromosome type aberration frequencies was 1.7:1. The aberrations of chromatid type were represented mainly by chromatid fragments (90.6%) whose level was 5.11 ± 0.75/100 cells, at average more than 5 times higher than in the control. In contrast to the control group, a distinctive feature of the spontaneous aberration spectrum was the appearance of dicentrics (0.16/100 cells) that cause reproductive cell death during one of the mitoses.

Obtained data show certain instability of the genome in PBL of CC patients even before the start of treatment and indicate the possibility for post-radiation complications. It is possible that the impact on the patient's organism caused by an oncological process may affect its sensitivity to radiation through genome modifications. Previous exposure to

COVID-19 may also be one of such modifying factors [51, 52].

Identification of key features of oxidation metabolism and genome instability in T-lymphocytes of patients with CC that are associated with cancer are essential for predicting an individual risk of post-radiation complications in the long term. Future work is needed to establish the possible role of studied indicators for the prevention of chemo-radiotherapy complications.

DISCLOSURE

The authors declare no conflict of interest.

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REFERENCES

1. **Wild CP, Weiderpass E, Stewart BW, eds.** World Cancer Report: Cancer research for cancer prevention. Lyon, France: International Agency for Research on Cancer; 2020. 611 p. https://digitallibrary.in.one.un.org/TempPdfFiles/5932_1.pdf. Accessed September 27, 2022.

2. **Fedorenko ZP, Sumkina OV, Horokh YEL, *et al.*** CANCER IN UKRAINE, 2020-2021: Morbidity, mortality, indicators activities of the oncology service. Bulletin of the National Cancer Registry Ukraine 2022; **23**: 129 p. (in Ukrainian).

3. **Lushnikova PA, Sukhikh ES, Izhevsky PV, *et al.*** Modern methods of radiation therapy for cervical cancer. Creative surgery and oncology 2021; **11**: 58–67. (in Russian). doi: 10.24060/2076-3093-2021-11-1-58-677

4. NCCN clinical practice guidelines in oncology: cervical cancer. National Comprehensive Cancer Network, version I.2018 — October 25, 2017. https://oncolife.com.ua/doc/nccn/Cervical_Cancer.pdf. Accessed September 27, 2022.

5. **Sarabhail T, Schaarschmidt BM, Wetter A, *et al.*** Comparison of 18F-FDG PET/MRI and MRI for pre-therapeutic tumor staging of patients with primary cancer of the uterine cervix. Eur J Nucl Med Mol 2018; **45**: 67–76. doi: 10.1186/s40644-020-00372-5

6. **Martinelli F, Signorelli M, Bogani G, *et al.*** Is aortic lymphadenectomy indicated in locally advanced cervical cancer after neoadjuvant chemotherapy followed by radical surgery? A retrospective study on 261 women. Eur J Surg Oncol 2016; **42**: 1512–8. doi: 10.1016/j.ejso.2016.06.004

7. **Meirow D, Nugent D.** The effects of radiotherapy and chemotherapy on female reproduction. Hum Reprod Update 2001; **7**: 535–43. doi: 10.1093/humupd/7.6.535

8. **Ye S, Yang J, Cao D, *et al.*** A systematic review of quality of life and sexual function of patients with cervical cancer after treatment. Int J Gynecol Cancer 2014; **24**: 1146–57. doi: 10.1097/IGC.0000000000000207

9. **Mkrtchian LS, Ivanov SA, Kulieva GZK, *et al.*** Quality of life of patients with cervical cancer after radiotherapy and chemoradiotherapy. Radiation Risk 2020; **29**: 120–8 (in Russian). doi: 10.21870/0131-3878-2020-29-1-120-128

10. **Han X, Yang Q, Zhang J, *et al.*** Correlation between changes in the number of peripheral blood lymphocytes and survival rate in patients with cervical cancer after radio-chemotherapy. Cancer Radiother 2021; **25**: 72–6. doi: 10.1016/j.canrad.2020.08.045

11. **Gavrilescu MM, Hutanu I, Ioanid N, *et al.*** Clinical value of hematological biomarkers in uterine cervical cancer. Chirurgia (Bucur) 2016; **111**: 493–9. doi: 10.21614/chirurgia.111.6.493

12. **Jeong MH, Kim H, Kim TH, *et al.*** Prognostic significance of pretreatment lymphocyte percentage and age at diagnosis in patients with locally advanced cervical cancer treated with definite radiotherapy. Obstet Gynecol Sci 2019; **62**: 35–45. doi: 10.5468/ogs.2019.62.1.35

13. **Armon-Omer A, Neuman H, Sharabi-Nov A, *et al.*** Mitochondrial activity is impaired in lymphocytes of MS patients in correlation with disease severity. Mult Scler Relat Disord 2020; **41**: 102025. doi: 10.1016/j.msard.2020.102025

14. **Takabayashi A, Kanai M, Kawai Y, *et al.*** Change in mitochondrial membrane potential in peripheral blood lymphocytes, especially in natural killer cells, is a possible marker for surgical stress on the immune system. World J Surg 2003; **27**: 659–65. doi: 10.1007/s00268-003-6926-7

15. **Georgescu SR, Mitran CI, Mitran MI, *et al.*** New insights in the pathogenesis of HPV infection and the associated carcinogenic processes: the role of chronic inflammation and oxidative stress. J Immunol Res 2018; **2018**: 5315816. doi: 10.1155/2018/5315816

16. **Zahra K, Patel S, Dey T, *et al.*** A study of oxidative stress in cervical cancer — an institutional study. Biochem Biophys Rep 2021; **25**: 100881. doi: 10.1016/j.bbrep.2020.100881

17. **Everett S.A., Wardman P**. Perthiols as antioxidants: radical-scavenging and pro-oxidative mechanisms. Methods Enzymol 1995; **251**: 55–69. doi: 10.1016/0076-6879(95)51110-5

18. **Scott D.** Chromosomal radiosensitivity, cancer predisposition and response to radiotherapy. Strahlenther Onkol 2000; **176**: 229–34. doi: 10.1007/s000660050005

19. **Ivankova VS, Domina EA, Khrulenko TV, *et al.*** Effects of brachytherapy on cytogenetic parameters and oxidative status in peripheral blood lymphocytes of gynecologic cancer patients. Exp Oncol 2021; **43**: 242–6. doi: 10.32471/exp-oncology.2312-8852.vol-43-no-3.16514

20. Product Information Histopaque®-1077 Hybri-Max™ (H8889). https://www.sigmaaldrich.com/ content/dam/sigma-aldrich/docs/ Sigma/ Product_Information_Sheet/2/h8889pis.pdf. Accessed October 17, 2019.

21. **Bokunyaeva NI, Zolotnitskaya RP**. Handbook of Clinical Laboratory Research Methods. M: Medicine, 1975. 384 p. (in Russian).

22. **Sivandzade F, Bhalerao A, Cucullo L.** Analysis of the mitochondrial membrane potential using the cationic JC-1 de as a sensitive fluorescent probe. Bio Protoc 2019; **9**: e3128. doi: 10.21769/BioProtoc.3128. www.bio-protocol.org/e3128. Accessed September 27, 2022.

23. MitoPT® JC-1 Assay Manual. Immuno-Chemistry Technologies, LLC. #F18-911-8-G, 8 p. https://cdn.shopify.com/ s/files/1/0549/4210/5739/files/911-924-mitopt-jc-1-assay-product-manual.pdf. Accessed September 27, 2022.

24. **Glavin OA, Domina EA, Mikhailenko VM, *et al.*** Metformin as a modifier of the oxidative state of peripheral blood and the viability of human lymphocytes under the influence of ionizing radiation. Oncologiya 2020; **22**: 84–91 (in Ukrainian). doi: 10.32471/oncology.2663-7928.t-22-1-2020-g.8855

25. **Liochev SI, Fridovich I.** Lucigenin (bis-N-methylacridinium) as a mediator of superoxide anion production. Arch Biochem Biophys 1997; **337**: 115–20. doi: 10.1006/abbi.1997.9766

26. **Druzhyna MO, Makovetska LI, Glavin OA, *et al.*** The free-radical processes in peripheral blood of patients with benign breast disease. Oncologiya 2018; **78**: 250–4. (in Ukrainian).

27. **Yao K, Wu W, Wang KJ, *et al.*** Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis 2008; **14**: 964–9.

28. **Tarpey MM, Wink DA, Grisham MB.** Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am J Physiol Regul Integr Comp Physiol 2004; **286**: R431–44. doi: 10.1152/ajpregu.00361.2003

29. **Serkiz YaI, Druzhina NA, Khriyenko AP, *et al.*** Blood Chemiluminescence Under Radiation Exposure. K: Naukova Dumka, 1989. 176 p. (in Russian).

30. **Olive PL, Banáth JP.** The comet assay: a method to measure DNA damage in individual cells. Nat Protoc 2006; **1**: 23–9. doi:10.1038/nprot.2006.5

31. **Mikhailenko VM, Muzalov II.** Exogenous nitric oxide potentiate DNA damage and alter DNA repair in cells exposed to ionizing radiation. Exp Oncol 2013; **35**: 318–24.

32. **Collins AR.** Investigating oxidative DNA damage and its repair using the comet assay. Mutat Res 2009; **681**: 24–32. doi: 10.1016/j.mrrev.2007.10.002

33. Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies. Vienna: IAEA, 2011. 232 p.

34. **Riccardi C, Nicoletti I.** Analysis of apoptosis by propidium iodide staining and flow cytometry. Nat Protoc 2006; **1**: 1458–61. doi: 10.1038/nprot.2006.238

35. **Hu ML.** Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol 1994; **233**: 380–5. doi: 10.1016/s0076-6879(94)33044-1

36. **Miwa S, Kashyap S, Chini E**, ***et al.*** Mitochondrial dysfunction in cell senescence and aging. J Clin Invest 2022; **132**: e158447. doi: 10.1172/JCI158447

37. **Song B, Li T, Chen S, *et al.*** Correlations between MTP and ROS levels of peripheral blood lymphocytes and readmission in patients with chronic heart failure. Heart Lung Circ 2016; **25**: 296–302. doi: 10.1016/j.hlc.2015.09.004

38. **Armon-Omer A, Neuman H, Sharabi-Nov A*, et al.*** Mitochondrial activity is impaired in lymphocytes of MS patients in correlation with disease severity. Mult Scler Relat Disord 2020; **41**: 102025. doi: 10.1016/j.msard.2020.102025. doi: 10.1016/j.msard.2020.102025.

39. **Anaya-Eugenio GD, Tan CY, Rakotondraibe LH, *et al.*** Tumor suppressor p53 independent apoptosis in HT-29 cells by auransterol from Penicillium aurantiacobrunneum. Biomed Pharmacother 2020; **127**: 110124. doi: 10.1016/j.biopha.2020.110124

40. **Zhu P, Luo W, Qian J, *et al.*** GSH/ROS Dual-responsive supramolecular nanoparticles based on pillar[6] arene and betulinic acid prodrug for chemo-chemodynamic combination therapy. Molecules 2021; **26**: 5900. doi: 10.3390/molecules26195900

41. **Wang Y, Yin S, Zhou Y, *et al.*** Dual-function of baicalin in nsPEFs-treated hepatocytes and hepatocellular carcinoma cells for different death pathway and mitochondrial response. Int J Med Sci 2019; **16**: 1271–82. doi: 10.7150/ijms.34876

42. **Perl A, Gergely PJr, Banki K, *et al.*** Mitochondrial dysfunction in T cells of patients with systemic lupus erythematosus. Int Rev Immunol 2004; **23**: 293–313. doi: 10.1080/08830180490452576

43. **Lee DS, Schrader A, Warchol M, *et al.*** Cisplatin exposure acutely disrupts mitochondrial bioenergetics in the zebrafish lateral-line organ. Hear Res 2022; **4:** 108513. doi: 10.1016/j.heares.2022.108513

44. **Banjerdpongchai R, Wudtiwai B, Sringarm K.** Cytotoxic and apoptotic-inducing effects of purple rice extracts and chemotherapeutic drugs on human cancer cell lines. Asian Pac J Cancer Prev 2014; **14**: 6541–8. doi: 10.7314/apjcp.2013.14.11.6541

45. **Goroshinskaya I; Popova N; Menshenina A, *et al.*** Free radical processes in the blood of patients with cervical cancer receiving various postoperative treatment modalities. Int J Gynecol Cancer 2019; **29**: A218. doi: 10.1136/ijgc-2019-ESGO.362

46. **Shah S, Kalal BS.** Oxidative stress in cervical cancer and its response to chemoradiation. Turk J Obstet Gynecol 2019; **16**: 124–8. doi: 10.4274/tjod.galenos.2019.19577

47. **Chernikova NV, Goroshinskaya IA, Frantsiyants EM, *et al.*** Intensity of free-radical reactions in metastasizing cervical cancer. Meeting abstract, 2021 ASCO annual meeting. J Clin Oncol 2021; **39***:* e17508. doi: 10.1200/JCO.2021.39.15_suppl.e17508

48. **Monaghan P, Metcalfe NB, Torres R.** Oxidative stress as a mediator of life history trade-offs: mechanisms, measurements and interpretation. Ecol Lett 2009; **12**: 75-92. doi: 10.1111/j.1461-0248.2008.01258.x

49. **Ott M, Gogvadze V, Orrenius S, *et al.*** Mitochondria, oxidative stress and cell death. Apoptosis 2007; **12**: 913-22. doi: 10.1007/s10495-007-0756-2

50. **Domina E.** Expediency on using radiomitigators in radiation therapy of cancer patients. Journal of science. Lyon. France 2020. **1**: 7–11. http://www.joslyon.com/wp-content/uploads/2020/08/Lyon_10_1.pdf. Accessed September 27, 2022.

51. **Chekhun VF, Domina EA.** Can SARS-CoV-2 change individual radiation sensitivity of the patients recovered from COVID-19? (Experimental and theoretical background). Exp Oncol 2021; **43**: 277–80. **doi:** 10.32471/exp-oncology.2312-8852.vol-43-no-3.16554

52. **Domina E.** Possible effects of the exposure to ionizing radiation on the patients recovered from COVID-19. ScienceRise: Biol Sci 2022; **1**: 4-7. doi: http://doi.org/10.15587/2519-8025.2022.254881

ОСОБЛИВОСТІ ОКИСНОГО МЕТАБОЛІЗМУ ТА ГЕНЕТИЧНІ ПОРУШЕННЯ У ЛІМФОЦИТАХ ПЕРИФЕРІЙНОЇ КРОВІ ПЕРВИННИХ ХВОРИХ НА РАК ШИЙКИ МАТКИ

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Стан питання: Рак шийки матки (РШМ), одне з найпоширеніших онкологічних захворювань у жінок, є глобальним викликом для системи охорони здоров'я як в Україні, так і у світі. Основним методом лікування пацієнток з місцево-поширеним РШМ є поєднана хіміо- та променева терапія, що може призвести до побічних ефектів у здорових клітинах та тканинах організму і, як наслідок, зниження ефективності лікування та якості життя хворих. Оцінка рівня пошкодження здорових радіочутливих клітин з оточення пухлини перед лікуванням є важливою для прогнозу та запобігання віддаленим наслідкам дії радіації. ***Мета:*** Дослідити окиснювальний метаболізм і генетичні порушення у лімфоцитах периферичної крові (ЛПК) первинних хворих на РШМ для оцінки можливості прогнозування променевих ускладнень на основі молекулярно-біологічних властивостей радіочутливих клітин крові. ***Матеріали та методи:*** Зразки периферичної крові 13 первинних хворих на РШМ $T_{1-4}N_{0-1}M_{0-1}$ використовували для виділення лімфоцитів та плазми і проведення комплексного дослідження окисного метаболізму (визначення мітохондріального трансмембранного потенціалу, супероксид аніон-радикалу ($O_2^{\cdot-}$), продукції активних форм кисню в ЛПК, а також рівня SH-груп у плазмі та про-/антиоксидантного співвідношення у гемолізатах). Розвиток генетичної нестабільності

оцінювали шляхом визначення двониткових пошкоджень ДНК, частоти і спектру хромосомних аберацій та апоптозу в ЛПК. ***Результати:*** У крові хворих на РШМ виявлено виражене підвищення інтенсивності генерації $O_2^{\cdot-}$ (1,5 раза), зменшення вмісту SH-груп (1,6 раза) та зсув про-/антиоксидантного балансу (1,4 раза) у бік прооксидантного компонента порівняно зі здоровими особами. Виявлені зміни, що пов'язані з розвитком оксидативного стресу, супроводжувалися підвищенням рівня двониткових розривів ДНК (у 2,1 раза), апоптозу (у 3,5 раза) і частоти клітин з хромосомними аберраціями (у 3,9 раза). Паралельно було виявлено значне зниження мітохондріального трансмембранного потенціалу (у 2 рази) та генерації рівня АФК у ЛПК (у 4 рази) хворих на РШМ. ***Висновок:*** Отримано попередні дані, які свідчать про порушення регуляції окиснювально-відновних процесів, зсув про-/антиоксидантного балансу в бік прооксидантної складової, що супроводжувалися розвитком генетичної нестабільності, зокрема підвищенням рівня пошкодження ДНК, аберацій хромосом та апоптотичної загибелі лімфоцитів крові у первинних хворих на РШМ.

Ключові слова: рак шийки матки, окиснювальний метаболізм, двониткові розриви ДНК, хромосомні аберації, апоптоз, лімфоцити периферичної крові.