
INFLUENCE OF SUPPORTING IMMUNOTHERAPY ON OXIDATIVE PROCESSES AND ANTIOXIDANT PROTECTION SYSTEM IN CERVICAL CANCER PATIENTS

Kamishov Sergey Viktorovich

*MD, PhD, Senior Researcher, department of chemotherapy
Republican Specialized Scientific and Practical Medical Center of Oncology and Radiology of the
Ministry of Health of the Republic of Uzbekistan, Tashkent*

Tillyashaykhov Mirzagaleb Nigmatovich

*Professor, Doctor of Medical Sciences,
Director of Republican Specialized Scientific and Practical
Medical Center of Oncology and Radiology of the
Ministry of Health of the Republic of Uzbekistan, Tashkent*

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ABSTRACT.

The indices of induced H₂O₂ chemical luminescence (CL), lipid peroxidation (LP) products and enzyme antioxidant protection (AP) system in the blood of 95 patients with cervical cancer of II-III stages who received concomitant extracorporeal immunopharmacotherapy (EIPT) were studied. All the patients received complex treatment which included polychemotherapy, surgery, and radiotherapy. The level of chemical luminescence serum in patients with cervical cancer was several times higher than the normal values. The content of lipid peroxidation products in red blood cells of patients and the activity of glutathione-dependent enzymes were initially high. After extracorporeal immunopharmacotherapy the intensity of those processes was significantly reduced. The immunotherapy has also increased the activity of catalase and superoxide dismutase.

In cervical cancer patients, the main disease and chemoradiation can increase the content of hydroperoxides in the body causing the imbalance in lipid peroxidation / antioxidant protection system. Extracorporeal immunopharmacotherapy as supporting therapy allows to reduce this imbalance significantly.

Keywords: antioxidant protection, lipid peroxidation, plasmapheresis, cervical cancer, extracorporeal immunopharmacotherapy (EIPT).

Introduction

As knowledge of the capabilities of the immune system in the fight against cancer began to deepen, treatment methods using the immune system against malignant tumors began to develop. Although immunotherapy of malignant tumors is a relatively new scientific trend, the results obtained during the last decade allow us to count on the important role of immunotherapeutic approaches in the treatment of oncological diseases [8,27].

Modern methods of extracorporeal immunopharmacotherapy (EIPT) are inherently an effective extension of therapeutic plasmapheresis. During PP the cellular elements are returned into the patient immediately after deplasma. During EIPT an additional release of the leukocyte fraction occurs which is then processed outside the body with a specific drug aimed at increasing or decreasing (depending on the disease) the functional activity of the participating inflammatory cells and immune reactions [16,21]. Free-radical oxidation of blood lipids is an important mechanism of maintaining the body homeostasis as it regulates the functioning of biological membranes [10]. The lipid peroxidation (LP) induction and regulation system includes the formation of activated oxygen metabolites such as superoxid anion radical, singlet oxygen, hydroxyl radical, hydrogen peroxide, etc. An adequate ratio of oxidative and antioxidant processes is a necessary condition for the body normal functioning [7]. The development of malignant neoplasms leads to significant changes in the lipid composition and intensity of LP reactions in the tumor and at the level of the organism as a whole. Numerous studies have shown the activation of free radical processes in the blood of cancer patients, accompanied by a decrease in

the content and activity of the antioxidant protection (AP) system. It is shown that the values AP system activity of the tumor, organs, and blood serum in the process of malignant growth change regularly and depend on the characteristics of the tumor and the state of the body. It was found that tumor tissue is able to accumulate natural antioxidants, as a result of which the tumor itself suppresses lipid peroxidation, and in normal tissues antioxidant protection decreases [5,13,26]. The intensity of free-radical oxidation in serum lipids shall be characterized by at least three criteria: oxidation, antioxidative activity, and oxidation rate. Oxidation is defined as the rate of initiation of primary free radicals and depends mainly on unsaturation of lipids. However, this ability does not always express the rate of oxidation in multicomponent systems. Lipids with high oxidability can show a low oxidation rate in the presence of a high concentration of antioxidants [15,28,29].

Induced chemiluminescence (CL) is an integral indicator of the LP condition in the interaction of substrates, catalysts and inhibitors of free-radical oxidation. It allows an *in vitro* assessment of the functional state of the body as a whole and can be used as an additional test in differentiated diagnostics of cancer diseases [28,29].

Today, cervical cancer (CC) remains one of the most common malignant tumours in women. Certain prognostically unfavourable systemic metabolic changes, such as the state of LP processes and the AP of cells are associated with the development of CC and, to a certain extent, contribute to the progression of tumour. Such disorders have not been sufficiently studied

yet [5,14,24-25]. At the same time, an excessive formation of free radicals is a known possible pathogenetic factor of carcinogenesis [13,20].

Various studies have shown an increased intensity of spontaneous CL of serum during esophageal, for estomach, lung or cervix cancer that was still growing in the absence of response to anticancer therapy [12,28]. The predominance of products of activated oxygen metabolites due to the increased formation or the depletion of antioxidants accompanied by the activation of destructive processes was called "oxidative stress" [26].

Thus, for many types of tumours, the activation of LP processes is an important pathogenetic factor adversely affecting the efficiency of treatment and the disease prognosis. The growth of malignancy increases the imbalance between the intensity of products of antioxidative enzymes, free-radical oxidation and the APS functional activity. Still, the impact of treatment on the LP processes in CC patients was not evidenced yet. These parameters are important for the study of the body-tumour interaction, the assessment of efficiency of treatment and the disease prognosis [17,19].

Purpose of the study was to assess the indices of induced CL, LP products and the enzymatic AP of serum in patients with stages II-III of CC after accompanying extracorporeal immunopharmacotherapy (EIPT).

Materials and methods

The study included 95 patients with clinical stages II-III of CC who received standard combined treatment in the oncogynecology department of the Republican Research Center of Oncology and Radiology of the Ministry of Health of the Republic of Uzbekistan during 2005-2014. All the patients had squamous cell carcinoma of the cervix histologically confirmed by the morphological analysis of surgical and biopsy material. The patients were aged 23 to 72 (mean age – 46.2±6.74 years).

All patients received complex treatment including polychemotherapy (PCT), surgery and radiotherapy (RT). In the first stage of treatment, the CC patients received total body or intra-arterial polychemotherapy in the regimen of: cisplatin 50 mg/m² + 5-fluorouracil 1000 mg/m², 4-6 courses of 4 days every 3 weeks, both in adjuvant and neoadjuvant regimen. The second stage included radical surgery or combined radiotherapy in radical regimen. The RT conducted at the third stage included remote tele-gamma-therapy (RTGT) and intracavitary brachytherapy. RTGT was conducted using "Theratron" or "AGAT-R" apparatus by split courses at 2 Gy ROD to 50 Gy SOD, 5 QW. The brachytherapy was performed every other day using "Gammamed" apparatus at 5 Gy ROD to 45÷55 Gy SOD eod [11].

According to immunotherapy methods as a part of complex treatment the patients were divided into the following groups:

Group 1 (control group) – 40 (42.1%) patients received no immunotherapy;

Group 2 – 30(31.6%) patients who were performed EIPT;

Group 3 – 25 (26.3%) patients received EIPT+plasmapheresis.

In Group 2 EIPT was performed by the exfusion of 200-250 ml of auto blood into "Gemakon" or "Terumo" sterile containers followed by the incubation with immune modulators such as Neovir in the total dosage of 750 mg (during 3 procedures), Cycloferon in the total dosage of 750 mg (during 3 procedures), or Polyoxidonium in the total dosage of 36 mg (for 3 procedures) at 37°C during 60-100 minutes, with the future return of the conjugate into the circulatory system of the patient.

Group 3 received EIPT as an extension of plasmapheresis (PP). The exfusion of 500-1000 ml of auto blood into "Gemakon" or "Terumo" sterile containers was followed by plasma extraction; separation of leukocytes, platelets and erythrocytes by centrifugation at 3000 rpm for 30 minutes; and the removal of 50-80 ml of buffy coat containing the antibodies, the circulating immune complexes, the cytokines, and cellular metabolism products. The buffy coat was incubated with immune modulators the same way as in Group 2, with the future return of the conjugate into the circulatory system of the patient.

Laboratory tests

Blood for serum was sampled from the ulnar vein of fasting patients. Blood serum was obtained by centrifugation of whole blood during 15 minutes at 1200 rpm. Induced CL was registered at the temperature of 37°C using electronic quantimeter consisting of the radiation detector FEU-38, the power source VS-22, the high-resistance milli voltmeter LPU-01 and the logger KSP-4 with a measurement range of 10 mV. Centrifuged serum was placed in a light-tight cubicle where CL was initiated with 1 ml of 1% H₂O₂ and recorded. The kinetic CL characteristics included: I_i – initial (fast) flash intensity; I_f – final CL intensity after 5 minutes of reaction, and ΣI₅– light-sum of LP reaction during 5 minutes of observation [10].

To determine the activity of antioxidant enzymes in erythrocytes, the latter were washed 3 times by 10-folds dilution of plasma with cold physiological saline with future repeated centrifugation at 2000 rpm and the temperature of 40°C. The haemoglobin hindering the determination of enzyme activity was precipitated by alcohol and chloroform mixture [1, 9].

The concentration of malondialdehyde (MDA) was determined according to L.I. Andreyeva [2]. The activity of superoxide dismutase (SOD) was determined by reducing the nitroblue tetrazolium reduction rate in the presence of reduced NAAD and phenazine methosulphate [12]. The diene conjugates (DC) level was assessed by the ratio of the intensity of absorption of isopropanol extracts at 215, 220 and 232 nm [22]. The catalase activity was determined according to A.I. Karpischenko based on the ability of hydrogen peroxide to form a stable coloured complex with molybdenum salts [18]. The activity of glutathione-S-transferase (GST) was determined according to A.I. Karpischenko by the rate of formation of glutathione-S-conjugates from reduced glutathione (G-SH) and 2,4-Dinitrochlorobenzene (DNCB)[13]. The activity of glutathione-S-peroxidase (GSP) was determined by the rate of utilization of hydrogen peroxide due to the oxidation of glutathione [6]. The activity of glutathione-S-

reductase (GSR) was determined according to Harutyunyan [4].

The studies were conducted in the cancer molecular chemistry laboratory in 2011-2014 (Head of laboratory – O.Yu. Balenkov, Candidate of Biology). The patients were lab examined before the immunotherapy and immediately before their discharge.

The statistical analysis was made using Statistics 6.0 software. The reliability of the differences between

two samples with a normal distribution of values was determined using Student's *f*-test.

Results and discussion

In the examined CC patients, the kinetics of induced CL was significantly increased several times vs. the normal level (Figure 1). The initial burst had the average amplitude of 324.8 ± 15.4 , and the final glow – 42.6 ± 2.4 , at the light sum of 148.4 ± 9.4 cu ($p < 0.05$).

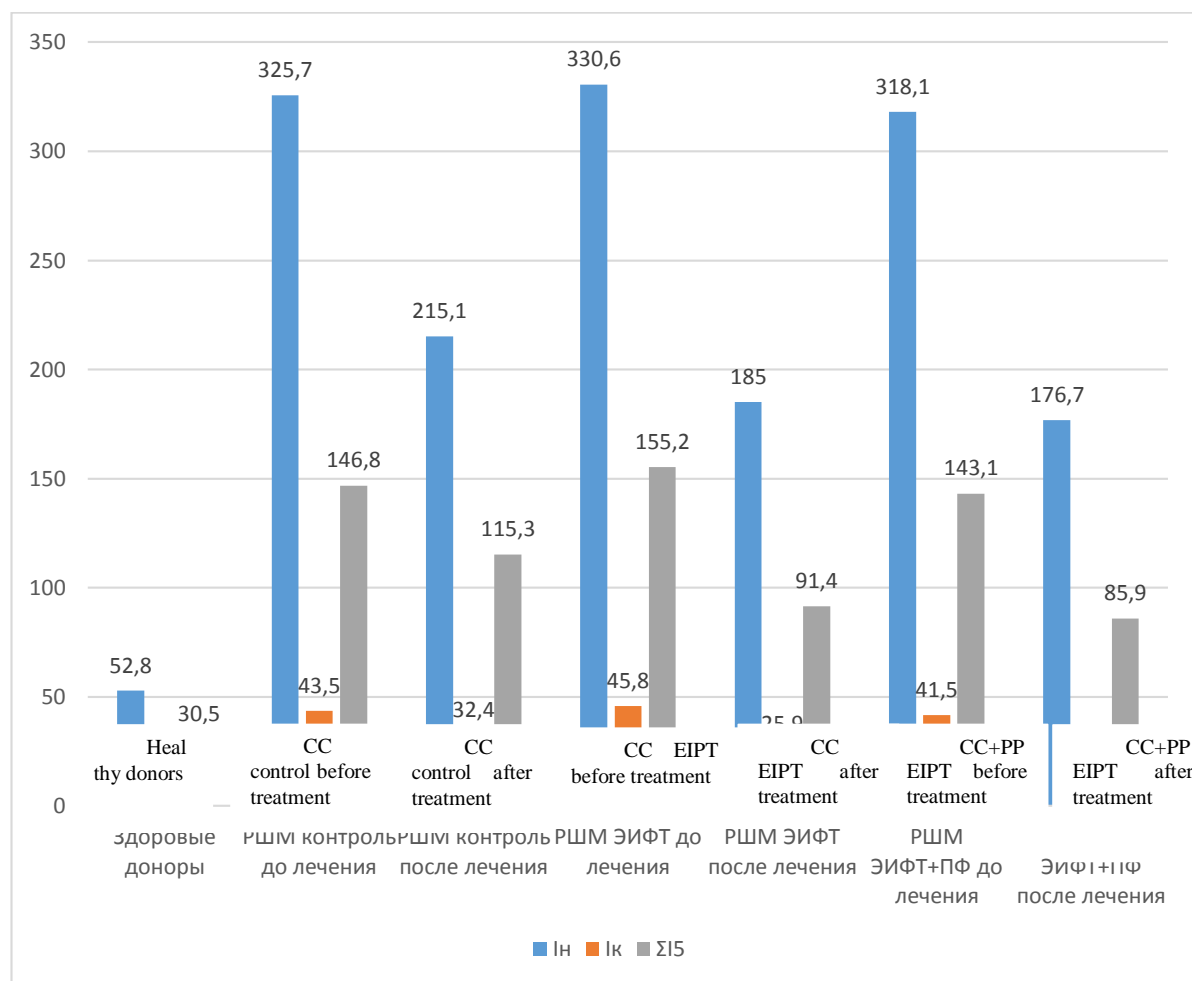


Fig 1. Induced H_2O_2 chemiluminescence in CC patients before and after treatment.

Complex therapy contributed to a significant decrease in luminescence. However, the luminescence indices did not return to normal values. The accompanying EIPT resulted in a more evident decrease in the studied indices of induced CL. The use of EIPT + PP was even more efficient probably due to the removal of metabolic products and toxins from blood plasma of the patients.

The initial burst amplitude evidences the interaction of transition metals in the serum – Cu, Fe and others – with organic and inorganic hydroperoxides. The

increased initial burst in CC patients could be associated with an increased level of lipid hydroperoxides in blood and the increased content of transition metals due to the disintegration of antioxidant enzymes.

CC patients had initially high intensity of LP processes judging by the content of its products, DC and MDA, in red blood cells (RBC) what was in line with the kinetics of induced CL. Its reduction in the Control group after combined therapy was not very pronounced. At the same time, the oxidative processes in RBC have significantly decreased in the groups of patients receiving EIPT, especially, EIPT + PP (Table 1).

Table 1 LP indices in the red blood cells of CC patients

Index	Groups of patients					
	Control group		EIPT		EIPT + PP	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
DC, mcM/L	528.7±24.6	475.2±22.0	536.7±26.7	276.4±16.7	530.7±21.9	254.4±12.4
MDA, mcM/L	415.1±21.5	380.6±19.2	410.1±21.3	220.6±10.4	421.1±20.6	180.8±12.3

Note: ($p < 0.01-0.05$)

The conducted studies have shown an initially increased level of activity of glutathione-dependent enzymes, GSR, GSP and GST, in CC patients against the reduced activity of SOD and catalase. Such behaviour of enzymatic member of AP system in malignant cells indicated the leading role of glutathione-dependent enzymes in inactivation of peroxides. In addition, the reduced activity of SOD and catalase could indicate a decrease in generation of H_2O_2 , inhibitor of cell multiplication, as CC progressed. An increase in the content of

GSR, GSP and GST with a simultaneous decrease in the activity of catalase and SOD enhanced the disproportion in the formation of O_2 and H_2O_2 .

After EIPT, the activity of GSR, GSP and GST was decreasing probably due to a decrease in the oxidant load thanks to a reduced content of hydroperoxides. On the contrary, the activity of catalase increased 2-3 times after immunotherapy while in the control group the increase was not significant (Table 2).

Table 2 Indicators of antioxidant protection in red blood cells of CC patients

Index	Groups of patients					
	Control		EIPT		EIPT + PP	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
SOD, cu	0.18±0.015	0.24±0.018	0.19±0.012	0.32±0.020	0.18±0.016	0.37±0.022
GSP, mM/GSH, min.	0.15±0.012	0.12±0.009	0.017±0.014	0.09±0.007	0.015±0.013	0.07±0.004
GSR, mcM/NADP*R, min.	0.09±0.007	0.07±0.006	0.08±0.006	0.05±0.004	0.09±0.007	0.04±0.002
GST, mcM/DNCB*GSH, min.	0.38±0.023	0.33±0.014	0.37±0.026	0.22±0.012	0.40±0.027	0.18±0.015
Catalase, mM/ H_2O_2 , min.	10.51±0.97	18.43±0.12	11.26±0.84	24.6±0.14	10.72±0.16	31.5±0.18

Note: ($p < 0.01-0.05$)

SOD was taken as a key enzyme of cell AP as it inactivated the superoxidation radical and worked in the cell in the cascade with catalase and GSP – the enzymes capable of decomposing hydrogen peroxide. After the immunotherapy, that indicator was much higher in comparison to the control group.

Certain prognostically unfavourable systemic metabolic changes, such as the state of LP processes and the AP of cells, are associated with the development of CC and, to a certain extent, contribute to the progression of tumour. Such disorders have not been sufficiently studied yet [3]. At the same time, an excessive formation of free radicals is a known possible pathogenetic factor of carcinogenesis [15, 26].

The observed dynamics of MDA and catalase in the control group indicated a disproportion in the formation of O_2 and H_2O_2 enhanced by an increased content of glutathione-dependent enzymes (GSR, GSP and GST) involved in the decomposition of hydroperoxides.

Discussion

When the tumour and the body interact, the system of nonspecific cellular immunity the malignant and cancer cells are not recognized as genetically homogeneous to the body. It produces a stress factor noted by many researchers and known as an "oxidative burst" which rapidly activates the membrane enzyme phospholipase A_2 . Phospholipase A_2 breaks the unsaturated higher fatty acids like arachidonic acid in the membranes of body tissues. Then the arachidonic acid is turned into prostaglandin D_2 which in turn activates the nonspecific cellular immunity (neutrophils, macrophages, etc.) with superoxidation radical presented in the active cell centre.

These substrates in the tumour carrier define the duration of the own antitumor protection of the body thus supporting the body's response to various tumour, infectious and other diseases. The formation of radicals in the body is opposed by the enzymatic system of regulation of LP products represented by superoxide dismutase, catalase, glutathione peroxidase, glutathione-

S-transferase and other natural antioxidants acting as non-enzymatic inhibitors of LP products [17,19].

In the conducted study the exceeded level of CL vs. the normal level of healthy donors indicated by a significant activation of oxidative processes. That could be due to the activity of nonspecific cellular immunity associated by activation of the reaction with a significant circulation of oxygen superoxidation radicals.

The efficiency of the conducted treatment was shown to correlate with the activity of free-radical oxidation of blood serum. The main disease and chemoradiation could increase the content of hydroperoxides in CC patients causing the imbalance in their LP/AP system. EIPT as accompanying therapy allowed a significant reduction of that imbalance.

Conclusion

The indices of induced CL of CC patients' blood serum were several times higher than normal values. At that, the level of LP processes in red blood cells of CC patients was initially high. EIPT methods effectively reduced the intensity of those processes.

The activity of glutathione-dependent enzymes in CC patients was initially high and tended to decrease after EIPT probably due to a decrease in the content of hydroperoxides.

The activity of catalase increased 2-3 times after immunotherapy while in the control group the increase was not significant. There was also a pronounced increase in SOD activity compared to the control group.

The observed dynamics of MDA and catalase in the control group evidenced a disproportion in the formation of O₂ and H₂O₂ enhanced by an increase in the content of glutathione-dependent enzymes (GSR, GSP and GST) involved in the decomposition of hydroperoxides.

EIPT as accompanying therapy allowed a significant reduction of the imbalance in the LP/AP system of patients with CC that could be caused by the main disease and chemoradiation that increased the content of hydroperoxides in the body.

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**СРАВНИТЕЛЬНАЯ СОЦИАЛЬНО - ДЕМОГРАФИЧЕСКАЯ И ПРОФЕССИОНАЛЬНАЯ
ХАРАКТЕРИСТИКА РУКОВОДИТЕЛЕЙ СЕСТРИНСКИМ ПЕРСОНАЛОМ ЛЕЧЕБНО-
ПРОФИЛАКТИЧЕСКИХ УЧРЕЖДЕНИЙ**

Кацова Галина Борисовна
Канд. мед. наук, доцент Оренбургского государственного медицинского университета

Попова Людмила Викторовна
Канд. мед. наук, доцент Оренбургского государственного медицинского университета

Дмитриева Марина Константиновна
Канд. мед. наук, доцент Оренбургского государственного медицинского университета

**COMPARATIVE SOCIAL - DEMOGRAPHIC AND PROFESSIONAL CHARACTERISTICS OF THE
MANAGERS OF MEDICAL AND PREVENTIVE INSTITUTIONS NURSE PERSONNEL**

Katsova Galina Borisovna
Candidate of Medical Sciences,
Associate Professor of Orenburg State Medical University

Popova Lyudmila Viktorovna
Candidate of Medical Sciences,
Associate Professor of Orenburg State Medical University

Dmitriev Marina Konstantinovna
Candidate of Medical Sciences,
Associate Professor of Orenburg State Medical University

АННОТАЦИЯ

Целью работы явилось сравнительное изучение социально-демографического и профессионального положения руководителей сестринским персоналом. Исследование проводилось методом анкетирования 234 медицинских сестер (47 главных и 187 старших медицинских сестер). Выявлены социально-экономические различия руководителей сестринским звеном различного уровня. Определена необходимость привлечения к управлению более молодых перспективных сотрудников.

ABSTRACT

The aim of the work is a comparative study of the socio-demographic and professional position of nursing staff managers. The study was conducted by questioning 234 nurses (47 main and 187 senior nurses). The socio-economic differences among the managers of the nursing unit at various levels have been revealed. The necessity to involve younger prospective employees in the management is determined.

Ключевые слова: медицинские сестры, профессиональная удовлетворенность, профессиональное выгорание.

Keywords: nurses, professional satisfaction, professional burnout.

В свете повышения роли медицинской сестры в процессе медицинского обслуживания населения, расширения функций среднего медицинского персонала назрела необходимость реорганизации сест-

ринского дела, которая позволила бы оптимизировать деятельность медицинских сестер, максимально использовать их профессиональный потенциал [1]. В процессе кадрового планирования руководителю медицинского учреждения приходится