R.J. Kerimova, Sh.M. Polukhova, M.B. Zulfugarova, F.E. Gulieva, A.F. Rustamova, E.M. Musayeva, Z.G. Aliyeva, A.V. Aslanova, N.A. Panakhova

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DETERMINATION OF BIOCHEMICAL MARKERS OF ALTERED HEPATIC METABOLISM IN WHITE RATS EXPOSED TO X-RAYS

The article presents information on a research study conducted to investigate changes in the levels of enzymes and free lipid peroxidation products during metabolic disorders in the liver of experimental animals exposed to X-rays. The study was carried out on 42 intact white rats, which were divided into three groups. The first group (control group) included 6 white rats. The second group consisted of 18 intact white rats that were irradiated with X-rays. In the third group, the levels of liver enzymes in the blood were measured 10 days after the cessation of X-ray exposure (18 animals). The levels of lipid peroxidation (LPO), malondialdehyde (MDA), diene conjugates (DC), hydrogen peroxide (H2O2), creatine phosphokinase (CPK), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined in the blood (in serum) of the experimental animals.

Keywords: X-rays, liver, biochemical markers

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Introduction. The liver is one of the largest and most vital organs in the human body, serving as the primary site for drug metabolism and detoxification. In addition to these roles, the liver is responsible for maintaining nutritional homeostasis, regulating cholesterol and glucose metabolism, and synthesizing clotting factors [8]. These essential functions make the liver particularly susceptible to the toxic effects of drugs and chemicals introduced through ingestion or other routes of exposure. Such toxic agents can impair the liver's detoxifying capacity by damaging functional macromolecules—such as lipids, proteins, and nucleic acids—via mechanisms that include free radical generation, antioxidant depletion, inflammation, and apoptosis. Structural damage in liver tissue may involve disruption of hepatocyte membranes and organelles, leading to cellular swelling, injury, and necrosis [5,11].

The liver is a highly active metabolic organ that is sensitive to various environmental factors. One such factor is ionizing radiation, which can cause severe damage or even death in living organisms when exposure occurs at relatively high doses due to its acute effects [7,11]. Moderately high doses of radiation may result in varying outcomes depending on factors such as the type of tissue exposed and the age at the time of exposure. In cancer radiotherapy, although the radiation is typically localized, surrounding healthy tissues including the liver may still receive high-dose exposure, potentially leading to acute damage such as fibrosis. In everyday life, humans are frequently exposed to radiation through medical procedures, including radiotherapy and diagnostic imaging. The effects of radiation are influenced by several biological variables such as species, age, and sex [6]. Animal studies have consistently shown that these variables including strain play a significant role in determining the severity of radiation-induced effects. Furthermore, lifestyle factors are critically important in human health and are believed to contribute to approximately 70% of cancer cases [9].

The aim of the study was to study changes in the level of enzymes and free products of lipid peroxidation in metabolic disorders in the liver of experimental animals exposed to X-rays.

Materials and methods. The experiments were conducted on white rats weighing 200-250 g, kept in a vivarium under normal conditions at the Scientific Research Center of Azerbaijan Medical University. During the study, we adhered to the guidelines set by the "European Bioethics Commission" (Strasbourg, 1986) and the local bioethics commission of "Azerbaijan Medical University". The rats were exposed to natural light, received a standard diet, and had free access to food and water.

The study was conducted on 42 intact white rats, which were divided into three groups. The first group (control group) consisted of 6 white rats. The second

Azerbaijan Medical University, Baku,Azerbaijan: **KERIMOVA R.J.** – Scientific Research Center,Doctor Neonatologist, Senior Researcher, Candidate of Medical Sciences, statya2021@mail.ru, ORCID: https://orcid.org/0009-0004-4323-9625; **POLUKHOVA Shakhzade M.** – Department of Pharmacology, Candidate of Pharmacological Sciences, Asst.Senior Lecturer, ORCID: https://orcid.org/0009-0009-4705-6363; **ZULFUGAROVA Mehriban B.** – Department of Pharmacognosy, Candidate of Biological Sciences ,Senior Lecturer, ORCID: https://orcid.org/0000-0003-1106-378X; **GULİEVA Firangiz E.** – Department of Biochemistry, Candidate of Biological Sciences, Associate Professor, ORCID: https://orcid.org/0000-0002-2206-1803; **RUSTAMOVA Afag A.** – Department of Normal Physiology, Candidate of Medical Sciences, Associate Professor. ORCID: https://orcid.org/0009-0003-9953-2276; **MUSAYEVA Elnura M.** – Department of Pharmacology, Candidate of Biological Sciences ,Assistant, ORCID: https://orcid.org/0000-0002-5807-901X; **ALİYEVA Zinyat G.** – Department of Physiotherapy and Medical Rehabilitation, Assistant, ORCID: https://orcid.org/0009-0002-5513-6717; **ASLANOVA Amina V.** – Department of Medical and Biological, Chief Laboratory Assistant, ORCID: https://orcid.org/0009-0007-3130-6044; **PANAKHOVA Nurangiz A.** – 1st Department of Internal Medicine, Doctor Cardiologist, Chief Laboratory Assistant, ORCID: https://orcid.org/0009-0000-0346-7325

group included 18 intact white rats that were irradiated with X-rays. In the third group, the amount of liver enzymes in the blood of the experimental animals was determined 10 days after the cessation of X-ray irradiation (18 animals).

The irradiation of the experimental animals with X-rays was performed using the "RUM-17" device. According to the recommendation of Eminov (2014), the radiation dose was 4 Gy, and the irradiation of animals was 1 Gy per day for 4 days [2].

- Intensity–180 kV;
- Current intensity–15 m;
- Filters–0.5 mm Cu+1.0 mm Al;
- Focal length factor–3
- Tubeless dose rate–0.86 Gy/sec

The experiments were conducted in all cases under pain-free conditions, and during the experiment, anesthesia was created by injecting 0.3 ml (50 mg/ml) of Calypsol solution into their abdominal cavity. As soon as the mentioned situation arose, the experimental animals were decapitated. After the irradiation was completed, biochemical examinations were conducted on the blood (in serum) samples taken from the experimental animals.

The levels of lipid peroxidation (LPO), malondialdehyde (MDA), diene conjugates (DC), hydrogen peroxide (H_2O_2), creatine phosphokinase (CPK), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined in the blood (in serum) of the experimental animals. [1,3].

The blood levels of the indicated markers were measured using reagent kits manufactured by Human on a fully automated BioScreen MS-2000 analyzer, made in the USA.

The concentration of hydrogen peroxide (H_2O_2), a product of free lipid peroxidation, was determined using the method of Askava T. and Matsushita S. (1980). The concentration of diene conjugates (DC), the intermediate product, was determined by the method developed by I.D. Stalnaya (1977).The concentration of malondialdehyde (MDA), the final product, was determined using the method proposed by Uchiyama and Michara (1978).

The determination of MDA (malondialdehyde), DK (diene conjugates) and H_2O_2 (hydrogen peroxide) analyses was performed using a BOECO S-300 (Boeckel & Co, Germany) spectrophotometer using reagent kits and methods. Malondialdehyde - TBA (thiobarbituric acid), diene conjugates - at a wavelength of 232-234nm, and hydrogen peroxide was measured using peroxide reagent kits.

The quantitative indicators of the obtained results were statistically analyzed based on modern recommendations (Lakin Q.F., 1990; Dodj M.S., 1997). For each group of animals under study, the following were calculated:

– mean value (M),
– standard error (m),
– minimum and maximum values;

Based on the obtained distributions, the following were used:

– for distributions close to normal (with ranking and symmetry of values),
– Wilcoxon-Mann-Whitney U-test — in cases where the data do not correspond to normal distribution.The choice of criteria was determined by the nature of the sample distribution, which meets the requirements of modern biomedical statistics. All calculations were performed in EXCEL tables at the Department of Medical Physics and Informatics of AMU.

A critical level of significance was considered at $p \le 0.05$.

The effect of X-rays (XR) leads not only to an increase in the concentration of enzymes in the blood, but also to enhanced free radical reactions of lipids in the liver tissue. In addition, lipid peroxidation (LPO) products such as hydrogen peroxide (H_2O_2), diene conjugates (DC), and malondialdehyde (MDA) were identified in the liver tissue. For example, while a sharp increase in DC concentration in the liver was observed in 100% of the experimental animals, the level of free LPO products in the liver remained unchanged. Hydrogen peroxide (H_2O_2) is not considered a direct product of lipid peroxidation. It participates in the initiation of free radical reactions by promoting the formation of hydroxyl radicals (•OH) through Fenton or Haber-Weiss reactions, which in turn lead to lipid peroxidation.

Results and discussion. The concentration of liver enzymes in the blood of white rats irradiated with X-rays differed from normal levels. The concentration of aspartate aminotransferase (AST) increased by 27% ($P<0.05$),alanine aminotransferase (ALT) increased by 30% ($P < 0.05$), gamma-glutamyl transferase (GGT) increased by 17%, and lactate dehydrogenase (LDH) increased by 31% ($P < 0.05$) compared to the intact state.

The concentration of the AST enzyme in the blood of white rats exposed to X-rays ranged from 28 to 50 U/l, with an average concentration of 37.4 ± 3.78 U/l. The concentration of the ALT enzyme ranged from 33 to 57 U/l, with an average concentration of 45.8 ± 4.53 U/l, which was higher than the intact state.

The concentration of gamma-glutamyl transferase in the blood of the experimental animals ranged from 33 to 60 U/l, with an average concentration of 50.6 ± 5.18 U/l. The concentration of the LDH enzyme varied between 320 and 390 U/l, with an average concentration of 482 ± 45.54 U/l.

It was found that X-rays, at the indicated dose, disrupted the physiological course of metabolism in the liver of white rats, significantly increasing the concentration of enzymes in the blood.Among these enzymes, the main indicator of the reparative process, creatine phosphokinase (CPK), increased the most. Its concentration in the blood was 52% higher than the level in the intact state ($P < 0.001$), ranging between 315 and 463 U/l, with an average concentration of 394.8 ± 24.25 U/l.As a result of the examinations conducted, we determined that the effects of X-rays (XR) not only increased the concentration of enzymes in the blood but also intensified the free radicalization of lipids in the liver tissue. The concentration of hydrogen peroxide (H_2O_2) varied between 3.25 and 4.25 ppm, with an average concentration of 3.75 ± 0.18 ppm. This represented an 87.5% increase compared to the intact state ($p < 0.001$). This increase was observed in 100% of the animals tested.

The concentration of diene conjugates (DC), an intermediate product of

Table 1

The amount of free lipid peroxidation products in the liver

Group	H_2O_2 (ppm)	DC (D232/ml)	MDA (nmol/mg)
Intact	2.00 ± 0.13	1.42 ± 0.12	1.14 ± 0.09
Group 2	2.75 ± 0.18*	2.30 ± 0.18**	2.94 ± 0.16***
Group 3	3.75 ± 0.18***	2.37 ± 0.16**	3.00 ± 0.14***

Note : * $P < 0.05$ – significant difference from intact group, ** $P < 0.01$ – highly significant difference, *** $P < 0.001$ – very highly significant difference

Table 2

Changes in the enzyme synthesizing function of the liver 10 days after stopping X-ray irradiation of experimental white rats

Group	AST (U/L)	ALT (U/L)	GGT (U/L)	LDH (U/L)	CPK (U/L)	ALP (U/L)
Group 1 (Control)	29.3 ± 2.0	35.2 ± 3.0	43.0 ± 3.5	367.0 ± 25.0	260.0 ± 15.0	234.0 ± 10.0
Group 2 (Post-X-ray)	37.4 ± 3.78 **	45.8 ± 4.53 **	50.6 ± 5.18 *	482.0 ± 45.54 **	394.8 ± 24.25 ****	302.0 ± 8.6 **
Group 3 (10 days later)	35.8 ± 3.38 *	43.2 ± 4.76 *	49.0 ± 4.55 *	467.0 ± 43.09 **	366.5 ± 21.80 ***	286.0 ± 12.08 *

Note : * P = 0.05, ** P < 0.05, *** P < 0.01, **** P < 0.001

free lipid peroxidation (LPO), ranged from 1.9 to 2.8 D232/ml, with an average concentration of 2.3 ± 0.16 D232/ml. This was 61.5% higher than the level in the intact state (p < 0.01). A sharp increase in the concentration of DC was observed in 100% of the animals tested. The concentration of malondialdehyde (MDA) increased significantly (158%) compared to the intact state (p < 0.001), ranging between 2.5 and 3.4 nmol/mg in the experimental animals. The average value was 2.94 ± 0.16 nmol/mg. The duration of free lipid peroxidation in the liver of white rats irradiated with X-rays is provided in Table 1.

Examinations were performed on white rats in group 3, 10 days after stopping irradiation. It was found that after the cessation of irradiation, the concentration of liver enzymes in the blood decreased moderately. The concentration of the AST enzyme varied between 28 and 46 U/l, with an average of 35.8 ± 3.38 U/l. Although the concentration of AST was 22% higher than the level in the intact state, it decreased by 4% compared to the irradiated animals (P = 0.05 in both cases).The concentration of the ALT enzyme in the blood taken from the white rats ranged from 30 to 56 U/l. The average concentration was 43.2 ± 4.75 U/l, which was 23% higher than the level in the intact state and 6% lower than the level in group 2 animals (P = 0.05 in both cases).

The concentration of gamma-glutamyl transferase (GGT) enzyme increased from 33 U/l to 58 U/l among the experimental animals. Its average concentration (M ± m = 49 ± 4.55 U/l) increased by 13% compared to the intact state.After stopping the irradiation, the concentration of LDH enzyme in the blood of white rats increased from 315 U/l to 575 U/l. Accordingly, the average concentration of LDH enzyme in the blood was 27% (P < 0.05) higher than the normal level, with a value of 467 ± 43.09 U/l. Compared to the animals in group 2, the concentration of LDH enzyme in the blood decreased by 3% (P = 0.05).The concentration of CPK enzyme in the blood of the white rats in the experiment ranged from 286 U/l to 415 U/l, with an average concentration of 366.6 ± 21.80 U/l. Compared to the intact state and group 2, the concentration of CPK in the blood of the experimental animals in group 3 was 41% higher than normal (P < 0.01). Compared to the level in the blood of the white rats in group 2, it decreased by 7% (P = 0.05).

The concentration of the alkaline phosphatase (ALP) enzyme ranged from 260 to 330 U/l, with an average concentration of 286 ± 12.08 U/l. It was 22% higher than the level in the intact state and 5% lower than the level in the blood of irradiated experimental animals (group 2) (P = 0.05 in both cases). The restoration of the enzyme-synthesizing function of the liver after exposure to X-rays is presented in Table 2.

Despite removing the subject from the radiation field, the liver function, which was disrupted by the effects of X-rays, cannot be fully restored [10,12].The results indicate that even after removing the experimental animals from the X-ray zone, the high intensity of free lipid peroxidation in the liver continues. As a result, 100% of the experimental animals in groups 2 and 3 exhibited higher-than-normal levels of free lipid peroxidation products in their livers [4].

Conclusion. X-ray radiation has a significant negative effect on the enzyme-synthesizing function of the liver. Even after cessation of irradiation, the liver function, impaired by X-ray exposure, is not fully restored. Thus, at a dose of 4 Gy (1 Gy per day for 4 days), animals experience symptoms of acute radiation sickness, such as weight loss, loss of appetite, diarrhea, lethargy, and general weakness. This dose is considered high enough to damage the hematopoietic system, which leads to a decrease in the number of leukocytes and platelets, as well as an increased risk of infections and bleeding.

The authors declare no conflict of interest.

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E.A. Krasavina, E.I. Choinzonov, D.E. Kulbakin, I.K. Fedorova, V.O. Tskhai, D.I. Novokhrestova, E.Yu. Kostyuchenko, S.D. Tomilina

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CRITERIA OF QUALITY OF LIFE FOR ASSESSING THE EFFECTIVENESS OF SPEECH REHABILITATION OF PATIENTS AFTER HEMIGLOSSECTOMY

KRASAVINA Elena Aleksandrovna – Candidate of Biological Sciences; speech therapist of the Department of Head and Neck Tumors; Cancer Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: krasavinaea@mail2000.ru, ORCID 0000-0002-8553-7039; **CHOINZONOV Evgeny Lhamatsyrenovich** – academician of the Russian Academy of Sciences, MD, Professor; head of the Department of Head and Neck Tumors; Cancer Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: info@tnimc.ru, ORCID: 0000-0002-3651-0665; **KULBAKIN Denis Evgenevich** – MD, senior researcher of the Department of Head and Neck Tumors; Cancer Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: kulbakin_d@mail.ru, ORCID 0000-0003-3089-5047; **FEDOROVA Irina Kazanovna** – junior researcher, head of the Department of Head and Neck Tumors; Cancer Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences, e-mail: irina_fedorova96@mail.ru, ORCID: 0000-0002-7540-8532; **TSKHAI Vladislav Olegovich** – PhD student of the Department of Head and Neck Tumors; Cancer Research Institute, Tomsk National Research Medical Center of the Russian Academy of Sciences; e-mail: tskhaivlad@mail.ru; ORCID: 0009-0003-3257-9002; **NOVOKHRESTOVA Daria Igorevna** – Ph.D. of Engineering Sciences, research associate, Laboratory of medical and biological research, Tomsk State University of Control Systems and Radioelectronics.(Tomsk, Russia); e-mail: ndi@fb.tusur.ru; **KOSTYUCHENKO Evgeniy Yuryevich** – Ph.D. of Engineering Sciences, associate professor, Head of Laboratory, Laboratory for the collection, analysis and management of biological signals, Tomsk State University of Control Systems and Radioelectronics.(Tomsk, Russia); email: key@fb.tusur.ru; **TOMILINA Svetlana Dmitrievna** – engineer, Department of Complex Information Security of Computer Systems. Tomsk State University of Control Systems and Radioelectronics.(Tomsk, Russia); email: tsd@fb.tusur.ru

The quality of life (QOL) of patients after radical surgery for oral cavity and oropharyngeal cancer is an important factor in determining the treatment effectiveness. Restoring functional and social capabilities, particularly speech and nutrition, is a crucial aspect of rehabilitation. **The purpose of the study** was to assess QOL of patients as one of the criteria for evaluating the effectiveness of speech rehabilitation technique using the OnkoSpeech v4.0 computer software. **Materials and methods**. The study included 140 patients with stage II–IV oral cavity and oropharyngeal cancer, who underwent hemiglossectomy (resection of ½ of the tongue). The median age of the patients was 54 years (range, 34 to 70 years, IQR-26). All patients were divided into two groups. Group I consisted of 70 patients who underwent speech restoration using the computer-software complex (OnkoSpeech v4.0 computer software). Group II comprised 70 patients who used the standard technique for speech restoration. To assess QOL, the EORTC QLQ-30 (version 3.0) and QLQ-H&N35 questionnaires were used. The assessment was carried before starting combination treatment, before starting rehabilitation, after completing rehabilitation, and 6 and 12 months after competing rehabilitation. **Results.** The analysis of data revealed significant differences in the parameters of speech restoration and quality of life between the groups. Group I (OnkoSpeech v4.0) patients demonstrated a statistically significant improvement in these parameters compared to group II patients ($p<0.05$). The EORTC QLQ-C30 scales did not show a statistically significant difference in the values of the functional scales (physical, role and social functioning) between the groups. According to the QLQ-H&N35 data, group I patients experienced less severe symptoms associated with speech, swallowing and social communication compared to group II patients. The standard technique (Group II) showed a slower recovery and less severity of positive dynamics. **Conclusion.** The use of OnkoSpeech v4.0 demonstrated higher efficiency of speech rehabilitation and improvement of patients' quality of life compared to the standard technique. The data obtained highlight the potential of integrating digital technologies into speech rehabilitation of cancer patients, which can be recommended for further implementation in clinical practice.

Key words: surgical treatment; functional disorders; speech rehabilitation; quality of life; computer software complex "OnkoSpeech v4.0".

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Introduction. Rehabilitation of patients after combination treatment for cancer is essential and growing trend in cancer care [1,6]. Evaluation of the effectiveness of rehabilitation techniques using the QOL criterion provides a deeper and more comprehensive understanding of their significance for patients [2,3,5]. The QOL assessment incorporates subjective patient-reported psychological, social, and functional aspects alongside objective clinical data to evaluate a rehabilitation technique's overall impact [4,7]. Comparison of QOL before and after using a new rehabilitation technology provides objective evidence of its effectiveness, allowing for a comparison with current methods. This approach helps ensure that patients receive the most suitable and effective treatments by evaluating how a new technology impacts their well-being [6,8].

The purpose of the study was to assess patients' QOL as one of the criteria