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РЕЦЕНЗИРУЕМЫЙ НАУЧНО-ПРАКТИЧЕСКИЙ

Южно-Российский онкологический журнал

Журнал входит в рекомендованный ВАК РФ перечень рецензируемых научных журналов и изданий для опубликования основных научных результатов диссертаций на соискание учёной степени кандидата и доктора наук.

«Южно-Российский онкологический журнал»: профессиональное медицинское издание. В нем публикуются новости медицинского и фармацевтического сообществ, научно-практические статьи для целевой аудитории – врачей-онкологов. Редакция журнала ставит своей задачей популяризацию научно-исследовательских работ и достижений онкологов Южного федерального округа, анализ процесса глубокой реорганизации здравоохранения в России. Редакция приглашает в качестве авторов всех, кто ищет и находит интересные решения многогранных задач, стоящих перед современной медициной, и хочет поделиться своими мыслями и наблюдениями с коллегами.

Цель: способствовать развитию онкологической медицины Юга России и внедрению её достижений в практику.

Задачи: освещать современные достижения онкологической службы Юга России; содействовать обмену опытом и передовыми знаниями между специалистами; информировать читателей об итогах крупных медицинских форумов.

В журнале размещаются публикации различных рубрик: обзоры литературы, мета-анализы, клинические исследования, наблюдения клинических случаев, обсуждения, анонсы и описания новых методов лечения.

Журнал принимает к публикации: оригинальные статьи, организации здравоохранения, лучевой диагностики, обмен опытом, обзоры, клинические наблюдения.

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South Russian Journal of Cancer: professional medical publication. It publishes news from the medical and pharmaceutical communities, scientific and practical articles for the target audience-oncologists. The editorial board of the journal aims to popularize the research works and achievements of oncologists of the Southern Federal District, to analyze the process of deep reorganization of healthcare in Russia. The editorial board invites as authors all those who are looking for and find interesting solutions to the multifaceted problems facing modern medicine and want to share their thoughts and observations with colleagues.

Purpose: to promote the development of cancer medicine in the South of Russia and the introduction of its achievements into practice.
Tasks: to highlight the current achievements of the oncology service in the South of Russia; to promote the exchange of experience and advanced knowledge between specialists; to inform readers about the results of major medical forums.
The journal contains publications of various categories: literature reviews, meta-analyses, clinical studies, observations of clinical cases, discussions, announcements and descriptions of new treatment methods.
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ORIGINAL ARTICLE

VARYING SEVERITY COVID-19 EFFECTS ON THE BLOOD INDICATORS OF INSULIN-LIKE GROWTH FACTORS FAMILY IN PATIENTS WITH NON-SMALL CELL LUNG CANCER

O. I. Kit, E. M. Frantsiyants, D. A. Kharagezov, V. A. Bandovkina✉, N. D. Cheryarina, Yu. A. Pogorelova, Yu. N. Lazutin, A. G. Milakin, I. A. Leyman, O. N. Stateshny

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ABSTRACT

Purpose of the study. An analysis of IGF and their carrying proteins levels in blood serum of patients with non-small cell lung cancer (NSCLC), depending on the severity of the previous COVID-19 infection.

Materials and methods. 60 patients with histologically verified NSCLC $T_{2-3}N_xM_0$ receiving treatment at the Thoracic Department (National Medical Research Centre for Oncology, 2020–2021), were included in the study. The control group included 30 NSCLC patients after asymptomatic or mild COVID-19 disease (15 men and 15 women); the main group included 30 (15 men and 15 women) patients after severe or moderate to severe COVID-19. The mean age of patients was 59.11 ± 2.89 years. Blood counts of donors of the same age were used as the norm.

Results. The levels of IGF-I, IGF-II, IGFBP2 and IGFBP3 in the blood serum of patients with NSCLC of the main and control groups were higher than those of donors by an average of 2.5, 2.1, 1.7 and 2.7 times, respectively ($p < 0.05$). The concentration of IGFBP1 was higher in the control group compared to the main group, and decreased in relation to donors: in the control in men and women by 1.4 and 1.9 times, and in the main group by 3.0 and 6.4 times, respectively ($p < 0.05$). The ratios of IGF and IGFBP1 increased in both groups: IGF-I/IGFBP1 – in the control group from 3.8 to 4.2 times, and in the main group from 7.9 to 14.4 times; IGF-II/IGFBP1 – in the control from 2.4 to 4.5 times, and in the main group from 6.6 to 12.7 times in men and women, respectively ($p < 0.05$).

Conclusions. The level of ligands and almost all of the studied carrier proteins, except for IGFBP1, increases in the blood of patients with NSCLC of both sexes, regardless of the severity of COVID-19. The ratio of IGF-I/IGFBP1 and IGF-II/IGFBP1 in the blood increases in both groups, most significantly in the group with severe and moderate COVID-19, which indicates excessive accumulation of IGF levels and may contribute to a more aggressive course of the malignant process.

Keywords: non-small cell lung cancer, COVID-19, IGF-I, IGF-II, IGFBP

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Compliance with ethical standards: the ethical principles presented by the World Medical Association Declaration of Helsinki, 1964, ed. 2013, were observed in the study. The study was approved by the Biomedical Ethics Committee at the National Medical Research Centre for Oncology (extract from the protocol of the meeting No. 7/111 dated 02/26/2021). Informed consent was received from all participants of the study.

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ОРИГИНАЛЬНАЯ СТАТЬЯ

ВЛИЯНИЕ COVID-19 РАЗЛИЧНОЙ СТЕПЕНИ ТЯЖЕСТИ НА ПОКАЗАТЕЛИ СЕМЕЙСТВА ИНСУЛИНОПОДОБНЫХ ФАКТОРОВ РОСТА В КРОВИ БОЛЬНЫХ НЕМЕЛКОКЛЕТОЧНЫМ РАКОМ ЛЕГКОГО

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РЕЗЮМЕ

Цель исследования. Изучить в сыворотке крови больных немелкоклеточным раком легкого (НМРЛ) содержание IGF и их белков-переносчиков в зависимости от степени тяжести перенесенного COVID-19.

Материалы и методы. В исследование включены 60 больных с гистологически подтвержденным НМРЛ стадии $T_{2-3}N_xM_0$, проходивших лечение в торакальном отделении ФГБУ «НМИЦ онкологии» Минздрава России с 2020 по 2021 гг. В контрольную группу вошли 30 больных раком легкого с бессимптомными или легкими случаями COVID-19 (15 мужчин и 15 женщин), в основную группу – 30 больных (15 мужчин и 15 женщин), перенесших болезнь в тяжелой или среднетяжелой форме. Средний возраст больных составил 59,11 ± 2,89 года. В качестве нормы использовали показатели в крови доноров того же возраста.

Результаты. В сыворотке крови больных НМРЛ основной и контрольной групп уровни IGF-I, IGF-II, IGFBP2 и IGFBP3 были выше значений доноров в среднем в 2,5, в 2,1, в 1,7 и в 2,7 раза соответственно ($p < 0,05$). Концентрация IGFBP1 была выше в контроле по сравнению с основной группой, а по отношению к донорам снижалась: в контрольной группе – у мужчин и женщин в 1,4 и 1,9 раза, а в основной – в 3,0 и 6,4 раза соответственно ($p < 0,05$). Коэффициенты соотношения повышались в обеих группах: IGF-I/IGFBP1 – в контрольной группе от 3,8 до 4,2 раза, а в основной от – 7,9 до 14,4 раза; IGF-II/IGFBP1 – в контрольной от 2,4 до 4,5 раза, а в основной группе – от 6,6 до 12,7 раза у мужчин и женщин соответственно ($p < 0,05$).

Заключение. У больных НМРЛ обоего пола в крови вне зависимости от тяжести перенесенного COVID-19, повышается уровень лигандов и почти всех исследованных белков-переносчиков, кроме IGFBP1. Соотношение IGF-I/IGFBP1 и IGF-II/IGFBP1 крови повышается в обеих группах, наиболее значимо в группе перенесших COVID-19 в тяжелой и среднетяжелой форме, что свидетельствует об избыточном накоплении уровня IGF в крови.

Ключевые слова: немелкоклеточный рак легкого, COVID-19, IGF-I, IGF-II, IGFBP

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено Комитетом по биомедицинской этике при ФГБУ «НМИЦ онкологии» Минздрава России (выписка из протокола заседания № 7/111 от 26.02.2021 г.). Информированное согласие получено от всех участников исследования.

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INTRODUCTION

The COVID-19 pandemic, caused by the appearance of severe acute respiratory syndrome 2 (SARS-CoV-2), has led to millions of cases and hundreds of thousands of deaths worldwide [1–3]. The biological sex of a person played a fundamental role in the heterogeneous outcomes of COVID-19 [4–6].

Among all types of cancer, lung cancer patients are of particular interest in the conditions of the COVID-19 pandemic, since the main primary focus of infection with the virus, the respiratory tract, is already at risk due to the presence of a tumor [7]. Previous outbreaks of respiratory viruses have shown that lung cancer patients are more vulnerable than other cancer patients because their pathological respiratory epithelium is probably more prone to rapid penetration of the virus into the lungs [8]. In fact, lung cancer is one of the most common types of cancer among cancer patients with COVID-19 [9; 10]. It is assumed that patients with lung cancer are at a higher risk of this severe form of COVID-19 [9; 11]. Recent studies have shown that the mortality rate of lung cancer patients is higher than that of the general population when infected with COVID-19 [12; 13]. However, comprehensive global data on the impact of the new coronavirus infection on the course of malignant disease in patients with lung cancer have not yet been presented.

Studies have shown that severe consequences in patients with COVID-19 do not develop directly due to the replication of SARS-CoV-2 itself, but are relatively associated with destructive inflammatory reactions caused by a huge activation of immune cells [14]. Therefore, an adjusted immune response is necessary to control and eliminate SARS-CoV-2 infection. On the other hand, uncorrected immune reactions and the production of a large number of inflammatory mediators and cytokines can have adverse and sometimes fatal consequences [15–17].

Insulin-like factor IGF-I and carrier protein IGFBP3 are most often associated with malignant lung tumors [18]. It is known that most of the circulating IGF-I is bound to the soluble plasma protein IGFBP3, while a very small percentage of IGF-I remains in an unbound, biologically active and accessible form. IGF-I can be "neutralized" after binding to IGFBP3, thereby suppressing its mitogenic and anti-apoptotic properties [19]. Thus, the IGF-I/IGFBP3 ratio is considered an indicator of IGF-I bioavailability. High serum IGF-I levels correlate with an increased risk of lung cancer, while high IGFBP3 levels are associated with a reduced risk of disease [20]. Moreover, in vitro studies have shown that IGF-1 promotes the growth and invasion of lung cells, which indicates the role of the IGF-I pathway in the oncogenic growth of non-small cell lung cancer (NSCLC) [21].

However, the significance of IGF-I and IGF-II as prognostic biomarkers has not yet been established. Based on these considerations, we sought to evaluate the serum levels of IGF-I, IGF-II and IGFBP1-3 in patients with NSCLC who had suffered COVID-19 of varying severity.

Purpose of the study: to elaborate the indicators of the system of insulin-like growth factors in the blood serum of patients with NSCLC who have suffered a coronovirus infection of varying severity.

MATERIALS AND METHODS

Prior to participation in the study, all patients received written informed consent. This study was approved by the Ethics Commission of the National Medical Research Centre for Oncology.

The study included men and women (60 people in total) with histologically or cytologically confirmed stage $T_{2-3}N_xM_0$ NSCLC, ECOG (PS) ≤ 2 working status, adequate organ function based on standard laboratory tests, including a complete blood count, serum biochemistry and coagulogram. The main exclusion criteria were previous treatment of NSCLC, type II diabetes mellitus, since it could affect IGF levels, and other concomitant neoplasms over the past five years, with the exception of non-melanoma skin carcinomas. The stage was determined according to the TNM classification. The step-by-step examination included computed tomography (CT) of the chest, abdominal cavity and brain. Bone scans were performed based on symptoms. All patients were examined before the start of treatment.

The control group included 30 patients (15 men and 15 women) with lung cancer with asymptomatic or mild cases of COVID-19, the main group included 30 patients (15 men and 15 women) those who have suffered COVID-19 in severe or moderate form. The age of the patients was: the control group of men 59.8 ± 1.67 (52–74); the control group of women 56.3 ± 3.98

(35–75); the main group of men 61.13 ± 1.82 (52–74); the main group of women 58.4 ± 3.73 (35–74).

According to the recommendations, a smear from the nasopharynx for COVID-19 PCR was obtained in all patients.

Collection of blood samples. Serum levels of IGF-I, IGF-II and IGFBP1,2,3 in peripheral blood were evaluated at the beginning of treatment. Blood samples were centrifuged at 5000 rpm for 10 minutes, then blood serum was collected and stored at –80 °C. Quantitative assessment of circulating levels of IGF-I, IGF-II and IGFBP1,2,3 was performed by enzyme immunoassay (Mediagnost, Germany). According to the recommendations available in the instructions for standard ELISA kits, each laboratory itself must determine the parameters of the norm characteristic of the examined population. The blood levels of IGF-I, IGF-II and IGFBP1,2,3 in conditionally healthy donors (20 men and 20 women) aged 40 to 75 years were taken as the norm. Since the donor studies were conducted in 2019, this contingent did not suffer from COVID-19 infection.

Statistical analysis. Statistical analysis was carried out using the Statistica 10 program. Normality was assessed using Kolmogorov-Smirnov methods, differences between groups were determined using the Student's t-test or the Mann-Whitney U-test, depending on the normality of the distribution. The value of $p < 0.05$ was considered as an indicator of statistical significance.

RESEARCH RESULTS

It was found that most of the indicators were higher in control group patients' blood serum than the values donors: in men, the level of IGF-I, IGF-II, IGFBP2 and IGFBP3 was increased by 2.7 times, 1.7 times, 2.1 times and 2.6 times, respectively, in women – by 2.2 times, 2.3 times, 1.4 times and 2.8 times, respectively (Table 1).

Only IGFBP1 in men and women of the control group was lower than the values in healthy donors by 1.4 times and 1.9 times, respectively. At the same time, the level of IGFBP1 was 1.4 times higher in the blood serum of men than in women and IGFBP2 was 1.5 times higher, and the level of IGF-II was 1.3 times higher in women than in men.

In the blood serum of patients of the main group, most of the indicators, as well as in patients of the

Table 1. Insulin-like growth factors and their carrier proteins in the blood of patients with lung cancer, depending on the severity of COVID-19

Groups	Sex	IGF-I ng/ml	IGF-II ng/ml	IGFBP1 ng/ml	IGFBP2 ng/ml	IGFBP3 ng/ml
Control	M	286.7 ± 19.7 $p^1 = 0.0000$	624.9 ± 51 $p^1 = 0.0020$ $p^2 = 0.0255$	8.56 ± 0.78 $p^1 = 0.0291$ $p^2 = 0.0479$	844.5 ± 63.2 $p^1 = 0.0000$ $p^2 = 0.0031$	3908.4 ± 277 $p^1 = 0.0000$
	F	230.1 ± 15.8 $p^1 = 0.0000$	839.8 ± 75.6 $p^1 = 0.0000$	6.22 ± 0.67 $p^1 = 0.0004$	551.4 ± 46 $p^1 = 0.0319$	4259.2 ± 415.4 $p^1 = 0.0000$
Main	M	274.9 ± 27.4 $p^1 = 0.0000$	775.7 ± 60 $p^1 = 0.0000$	3.96 ± 0.24 $p^1 = 0.0000$ $p^2 = 0.0000$ $p^3 = 0.0000$	638.6 ± 76.8 $p^1 = 0.0142$	3811.9 ± 563.6 $p^1 = 0.0003$
	F	240.7 ± 45.3 $p^1 = 0.0079$	720.7 ± 65.3 $p^1 = 0.0003$	1.9 ± 0.16 $p^1 = 0.0000$ $p^3 = 0.0000$	601.7 ± 66.1 $p^1 = 0.0208$	4196.6 ± 421.6 $p^1 = 0.0000$
Normal values		106.2 ± 12.5	360.6 ± 58.6	12.1 ± 1.3	405 ± 45.7	1502.4 ± 59.8

Note: statistically significant in relation to: [1] – to the indicator in donors; [2] – to the indicator in women in the corresponding group; [3] – to the corresponding indicator in the control group.

control group, were higher than the values of conditionally healthy donors. In the blood serum of men of the main group, the level of IGF-I, IGF-II, IGFBP2 and IGFBP3 was increased 2.6 times, 2.2 times, 1.6 times and 2.5 times, respectively, in women – 2.3 times, 2 times, 1.5 times and 2.8 times, respectively. Also, as in patients of the control group, in the main group, the level of IGFBP1 was lower in donors: in men by 3.1 times, in women by 6.4 times. And only by this indicator there were differences between the control and the main groups: the level of the carrier protein in men of the control group was 2.2 times higher than in the blood serum of men of the main group, in women – by 3.3 times (Table 1).

According to some indicators, we found no fundamental differences in the blood of patients of the control and main groups groups, although all the studied indicators had significant differences from the values in healthy donors.

Further, it was of interest to investigate the ratios of IGF and carrier proteins, since it is known that most of the circulating IGF is associated with soluble plasma IGFBP proteins, while a small percentage of IGF remains in an unbound and biologically active form. The results are presented in table 2.

It was found that the ratio of IGF-I/IGFBP1 and IGF-II/IGFBP1 in the blood serum of male patients of the control group was 3.8 times and 2.4 times higher than in healthy donors, respectively, and in the blood of women – 4.2 times and 4.5 times, respectively. Even more pronounced changes were found in the blood serum received from the main group: the ratio of IGF-I/IGFBP1 and IGF-II/IGFBP1 was higher than in healthy donors by 7.9 times and 6.6 times, respectively, and in women – by 14.4 times and 12.7 times, respectively. I.e. the ratio of IGF-I/IGFBP1 and IGF-II/IGFBP1 in the blood was significantly higher in patients who underwent Covid-19 in severe and moderate form: in men – 2.1 times and 2.7 times, respectively, in women – 3.4 times and 2.8 times, respectively, compared with patients of the control group (Table 2).

Thus, in the blood serum of lung cancer patients of both sexes, regardless of the severity of COVID-19, there is an increase in the level of ligands and almost all of the studied carrier proteins, except IGFBP1, a family of insulin-like growth factors with an increase in the ratio of IGF-I/IGFBP1 and IGF-II/IGFBP1. The transmitted infection of COVID-19 in severe and moderate form further aggravates the level of the IGF-I/

Table 2. Ratios of insulin-like growth factors to carrier proteins in the blood of patients with lung cancer, depending on the severity of COVID-19

Groups	Sex	IGF-I/ IGFBP1	IGF-I/ IGFBP2	IGF-I/ IGFBP3	IGF-II/ IGFBP1	IGF-II/ IGFBP2	IGF-II/ IGFBP3
Control	M	33.5 ± 1.7 p^1 = 0.0000	0.34 ± 0.02	0.07 ± 0.005	73 ± 2.6 p^1 = 0.0000 p^2 = 0.0000	0.74 ± 0.04	0.16 ± 0.008
	F	37 ± 5.6 p^1 = 0.0000	0.42 ± 0.06	0.05 ± 0.009	135 ± 5.2 p^1 = 0.0000	1.32 ± 0.09	0.2 ± 0.01
Main	M	69.4 ± 4.7 p^1 = 0.0000 p^2 = 0.0016 p^3 = 0.0000	0.43 ± 0.05	0.07 ± 0.02	195.9 ± 10.2 p^1 = 0.0000 p^2 = 0.0002 p^3 = 0.0000	1.21 ± 0.11	0.2 ± 0.05
	F	126.7 ± 12.8 p^1 = 0.0000 p^3 = 0.0000	0.4 ± 0.04	0.06 ± 0.02	379.3 ± 49.5 p^1 = 0.0000 p^3 = 0.0000	1.2 ± 0.14	0.17 ± 0.03
Normal values		8.8 ± 1.3	0.36 ± 0.009	0.07 ± 0.006	29.8 ± 3.8	0.9 ± 0.08	0.24 ± 0.03

Note: statistically significant in relation to: [1] – to the indicator in donors; [2] – to the indicator in women in the corresponding group; [3] – to the corresponding indicator in the control group.

IGFBP1 and IGF-II/IGFBP1 ratio, which leads to an increase in free, biologically active IGF.

DISCUSSION

The IGF pathway is a complex, multilevel system of ligands, receptors, carrier proteins and cellular signaling cascades with several levels of regulation. In a broad sense, the IGF family modulates cell behavior through endocrine, paracrine, and autocrine pathways [22]. Binding of IGF complexes to their corresponding receptors induces cellular adaptation, which promotes survival, proliferation and invasion under normal conditions of human physiology and in many types of cancer [23].

Two insulin-like growth factors, i.e. IGF-I and IGF-II, have been identified. Although IGF-II is assumed to regulate differentiation and survival of fetal musculoskeletal cells, understanding of its mechanisms of action is limited. At the same time, its molar ratio with respect to IGF-I in adults is 3:1. Since the unfavorable expression of IGF-II can affect a number of metabolic conditions, it is assumed that IGF-II continues to affect tissues throughout life [24]. Studies have also shown the involvement of IGF-II in phenotypic plasticity, potentially leading to more aggressive and resistant clones in progressive tumors [25]. The study of IGF-II in this context is a very active and developing research topic. On the contrary, information about the function of IGF-I revealed a link between a violation of its regulation and oncogenesis [22].

In this study, we found that in the blood serum of lung cancer patients, regardless of the severity of COVID-19, most indicators of the system of insulin-like growth factors and their carrier proteins were elevated, and regardless of the gender of the patients. The exception was the IGFBP1 protein, the level of which in the blood of patients was reduced, especially in patients of the main group.

Regarding IGF-I, our results confirm studies [20] that have shown that high levels of IGF-I in blood serum correlate with an increased risk of lung cancer. And the study [26] was the first to evaluate serum IGF-I levels in patients with severe COVID-19 compared to healthy people. The results showed that serum IGF-I levels in patients with severe COVID-19 did not differ significantly compared to healthy subjects. Therefore, it is logical to assume that the increase in the level of indicators of the system of insulin-like factors is the result of the presence of NSCLC in patients and is not associated with the infection. It is now well known that subjects in the general population whose serum IGF-I levels are at the upper limit of the normal range are at increased risk of developing several types of cancer [27].

Proteins binding insulin-like growth factor (IGFBPs) 1–6 bind IGF, but not insulin with high affinity. Initially, they were identified as serum carriers and passive inhibitors of IGF action. However, subsequent studies have shown that IGFBPs not only inhibit the actions of IGF, but in many cases they also enhance these actions. IGFBPs are widely expressed in most tissues and are flexible endocrine and autocrine/paracrine regulators of IGF activity, which is necessary for this important physiological system. More recently, it has been established that individual IGFBPs have IGF-independent action [28].

In this study, it was shown that the men of the control group had higher blood serum levels of IGFBP1 and IGFBP2, but not IGFBP3, compared with women, i.e. there were gender differences. In the blood of patients of the main group, gender differences were preserved only for the IGFBP1 protein. At the same time, in patients of both sexes of the main group, a decrease in the level of IGFBP1 was found relative to the indicators in the blood of the control group of patients.

Currently, there is interest in IGFBP1, namely its IGF-dependent and independent action from the position of influencing insulin sensitivity [29]. A completely different interest is shown in the IGFBP3 protein, which is the main circulating IGFBP, and also has an IGF-independent effect in response to DNA damage and EGF signaling. It has been found that the expression of recombinant human IGFBP3 (rhIGFBP3) blocks the IGF-dependent action of IGFBP3, and it has been shown that it has anti-cancer activity in vitro and in vivo [30]. However, a recent study of 11 gastric cancer cell lines showed that IGFBP1 expression levels were extremely low in all cell lines [31]. IGFBP1 is also known to block DNA synthesis, cell growth and differentiation, and also enhances the action of IGF-I in combination with certain reagents, such as platelet-depleted plasma, or in certain cell lines [22]. In [32], there was a tendency to reduce the risk of lung cancer with an increase in the concentration of IGFBP1 in serum, but these results did not reach statistical significance.

Insulin is the main regulator of IGFBP1, inhibiting its synthesis in the liver and other tissues, and the observed tendency to reverse the risk of lung cancer with IGFBP1 may be a consequence of higher insulin levels. The physiological relationship between IGFBP1 and insulin is reflected in the authors' data, which showed an inverse correlation of insulin with the concentration of IGFBP1 in the blood. Only one study has linked IGFBP1 to poor overall survival in lung adenocarcinoma [33].

It is known that an increase in the ratio between IGF and IGFBP reflects an increase in free, biologically active IGF, therefore, treatments that reduce this ratio have significant appeal as cancer prevention tools in people without cancer and with cancer relapses [34]. IGFBPs modulate cell proliferation, survival, differentiation, migration, and invasion. The IGFBP family provides an additional, predominantly extracellular mechanism for regulating IGF activity. A distinctive feature of IGFBPs is their binding to IGF-I and IGF-II, but not to insulin with high affinity. In most cases, they inhibit the actions of IGF, preventing binding to IGF receptors, but they can also enhance their action. IGF-independent actions of IGFBP have also been described over the past two decades. More recently, they have also been shown to regulate aging and autophagy, as well as angiogenesis. Due to these cellular effects, IGFBPs are involved in a number of physiological and pathological processes, including processes underlying metabolism, immune regulation, cancer and neurological diseases [28].

We studied the ratio of IGF and IGFBP in the blood serum of patients of the control and main groups. It turned out that only IGF-I/IGFBP1 and IGF-II/IGFBP1 were elevated. Moreover, these ratios had not only some gender differences, but also depended on the severity of Covid-19: if only the IGF-II/IGFBP1 ratio had gender differences in the control group, then in the main group both IGF-I/IGFBP1 and IGF-II/IGFBP1 in women exceeded the values in the blood of men.

In the literature, we have not found similar data in oncopathology. An increase in IGF-I and the binding proteins IGFBP-1 and IGFBP3 was recorded in damage and death of lung epithelial cells, as well as in early acute respiratory distress syndrome (ARDS), while a decrease in IGF-1 and IGFBP3 was reported in the late stages of ARDS [35]. As a rule, IGFBP3 is the most common IGFBP in the blood and acts as a transport workhorse for the IGF protein superfamily [35]. Consequently, its main IGF-dependent function is to control the amount of free IGF in circulation, which provides numerous subsequent effects, including dosing IGF for cell proliferation or enhancing apoptosis.

CONCLUSION

Thus, regardless of the severity of COVID-19, the blood serum levels of ligands and almost all of the studied carrier proteins, increases in NSCLC patients of both sexes, except IGFBP1. The IGF-I/IGFBP1 and IGF-II/IGFBP1 blood ratios increase in both groups, however most significantly in the group of severe and moderate form COVID-19 survivors, which indicates an excessive accumulation of IGF levels in the blood. IGF-system biomarkers may be useful in screening, prognosis and treatment of lung cancer, although their exact application requires further research.

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ORIGINAL ARTICLE

LEVELS OF BIOGENIC AMINES IN LUNG TISSUES OF PATIENTS WITH NON-SMALL CELL LUNG CANCER AFTER COVID-19 OF VARIOUS SEVERITY

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ABSTRACT

Purpose of the study. Was to analyze levels of biogenic amines (serotonin and its metabolite 5-HIAA, dopamine, norepinephrine and histamine) in lung tissues of patients with lung cancer with previous COVID-19 infection.
Patients and methods. The study was carried out on samples of intact lung tissues, tumor tissues and peritumoral lung tissues obtained during open biopsy while performing radical surgery from patients with morphologically verified non-small cell lung cancer (NSCLC), stage I–IIIA ($cT_{1-3}N_xM_0$). The main group included 30 NSCLC patients (15 men and 15 women) after severe or moderate to severe COVID-19 who required hospitalization. The control group included 15 men and 15 women with NSCLC after asymptomatic or mild SARS-CoV-2 infection. The mean age of patients was 59.11 ± 2.9 years. Levels of dopamine, norepinephrine, serotonin, 5-hydroxyindoleacetic acid (5-HIAA) and histamine were measured by ELISA (IBL, Germany).
Results. All studied lung tissue samples from men and women of the main group, compared to the control group, showed deficiency of catecholamines with their ratio unchanged, and changes in serotonin metabolism to ensure its stable level. Thus, levels of dopamine in samples of patients of the main group were lower on average by 1.3 times, norepinephrine by 1.3–3.3 times, serotonin by 1.6 times, and 5-HIAA by 1.8–4 times. At the same time, sex differences were observed in histamine levels. Regardless of the COVID-19 severity, levels of diamine in women were lower in the resection line tissue by an average of 2.4 times, and in the peritumoral tissue by 1.6 times, compared with men, but there were no sex differences in the tumor tissue.
Conclusion. Apparently, changes in the levels of dopamine, norepinephrine, and serotonin in lung tissues could be associated with the severity of SARS-CoV-2 infection. Since dopamine is involved in counteracting the carcinogenic action of the adrenergic system and in the regulation of various immunocompetent cells in the tumor microenvironment, such changes in the biogenic status in the lungs of patients of the main group could lead to a more severe tumor course.

Keywords: dopamine, serotonin, histamine, lung cancer, COVID-19

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Compliance with ethical standards: The ethical principles presented by the World Medical Association Declaration of Helsinki, (1964, ed. 2013) were observed in the study. The study was approved by the Ethics Council of the NMRC for Oncology of the Ministry of Health of the Russian Federation (extract from the protocol of the meeting No. 6 dated 01/17/2022). Informed consent was received from all participants of the study.

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3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

УРОВЕНЬ БИОГЕННЫХ АМИНОВ В ТКАНИ ЛЕГКОГО БОЛЬНЫХ НЕМЕЛКОКЛЕТОЧНЫМ РАКОМ ЛЕГКОГО ПЕРЕНЕСШИХ COVID-19 РАЗЛИЧНОЙ СТЕПЕНИ ТЯЖЕСТИ

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РЕЗЮМЕ

Цель исследования. Изучение содержания биогенных аминов – серотонина и его метаболита 5-ОИУК, дофамина, норадреналина и гистамина в тканях легкого у больных раком легкого перенесших COVID-19 различной степени тяжести.
Пациенты и методы. Для исследования послужили образцы легочной ткани вне зоны опухолевого роста, ткани опухоли и ее перифокальной зоны, полученные в результате открытой биопсии при выполнении радикальных операций у больных морфологически верифицированным немелкоклеточным раком легкого (НМРЛ) I–IIIA стадии ($cT_{1-3}N_XM_0$). В основную группу вошли 30 больных НМРЛ (15 мужчин и 15 женщин), перенесших COVID-19 в тяжелой и средней тяжести форме, потребовавшей госпитализации, контрольную группу аналогично составили 15 мужчин и 15 женщин с НМРЛ, у которых инфекция SARS-CoV-2 протекала бессимптомно или в легкой форме. Средний возраст пациентов составил 59,11 ± 2,9 года. Количественную оценку содержания дофамина, норадреналина, серотонина, 5-оксииндолуксусной кислоты (5-ОИУК) и гистамина выполняли методом ИФА (IBL; Германия).
Результаты. Во всех исследованных образцах тканей легкого мужчин и женщин основной группы, по сравнению с контрольной группой, имеет место недостаточность катехоламинов, без нарушения их соотношения и изменение метаболизма серотонина для обеспечения его устойчивого уровня. Так, уровень дофамина в образцах пациентов основной группы был ниже в среднем в 1,3 раза, норадреналина в 1,3–3,3 раза, серотонина в среднем в 1,6 раза, а 5ОИУК в 1,8–4 раза. В тоже время установлены половые различия в содержании гистамина, когда у женщин, вне зависимости от тяжести перенесенного COVID-19 уровень диамина был ниже в линии резекции в среднем в 2,4 раза, а в перифокальной зоне в 1,6 раза, по сравнению с мужчинами, но отсутствие половых различий в ткани опухоли.
Заключение. Очевидно, изменения уровня дофамина, норадреналина и серотонина в тканях легкого могут быть связаны с тяжестью перенесенной инфекции SARS-CoV-2. Учитывая то, что дофамин участвует в противодействии канцерогенному эффекту адренергической системы и в регуляции различных иммунокомпетентных клеток в микроокружении опухоли, подобные изменения биогенного статуса в легком у больных основной группы могут приводить к более тяжелому течению злокачественного процесса.

Ключевые слова: дофамин, серотонин, гистамин, рак легкого, COVID-19

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BACKGROUND

Coronavirus disease (COVID-19) is caused by a new virus – severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1–3], which can be transmitted from one person to another. By January 3, 2022, more than 290 million people worldwide had been diagnosed with COVID-19, and more than 5.4 million of these patients had died [4]. Lung cancer (LC) ranks second among the most common malignant neoplasms in the world in terms of morbidity and mortality [5–7]. Patients with LC may be at a higher risk of infection with SARS-CoV2 and have a worse prognosis for the course of the disease [8]. As a rule, multiple immune disorders are observed in patients with LC [9], which may affect the effectiveness of COVID-19 treatment.

There is still insufficient information on the impact of COVID-19 infection on the survival of patients with-LC, as well as on complications associated with infection and prognostic factors. In a meta-analytical study conducted by Desai A. D. and co-authors (2022) [10], with data on 2,922 cancer patients at various stages hospitalized with COVID-19, it was found that male gender, middle age and recent active cancer therapy were factors associated with mortality. Other authors note that in addition to age and chronic concomitant diseases, the need for invasive or non-invasive mechanical ventilation and female gender are identified risk factors for respiratory long-term complications of COVID-19 [11].

Data on subacute and long-term consequences of COVID-19 are accumulating. The most severe manifestation of post-infectious lung damage is pulmonary fibrosis, which has a profound long-term effect on the respiratory health of patients. More than a third of patients who survived severe COVID-19 pneumonia developed pulmonary fibrosis [12]. In animal models, SARS-CoV-2 infection also induced the expression of profibrotic cytokines and the occurrence of pulmonary fibrosis [13]. Although the specific mechanism requires further study, lung fibrosis caused by SARS-CoV-2 has much in common with lung fibrosis caused by other viruses.

It is worth noting that pulmonary fibrosis after a viral infection may not be a direct result of infection, and other factors, such as trauma caused by ventilation, may also play a role. Many severe patients with viral infection undergo artificial lung ventilation (AVL) during treatment, and available studies have shown that patients with a longer period of ventilation had a higher probability of developing pulmonary fibrosis and a worse prognosis [14]. Diem K. et al. (2020) showed that alveolar cells under mechanical stress can produce signaling molecules to communicate with neighboring cells and promote a fibrous reaction [15].

The mechanisms underlying the pulmonary fibrosis caused by the virus need to be studied in depth. Research can help doctors assess the risks of a combination of COVID-19 and lung cancer, which can ultimately reduce or even prevent deaths. A key gap in knowledge and acute unmet medical need in the clinical management of lung cancer patients in the era of the COVID-19 pandemic is the characterization of the molecular mechanisms linking these two diseases. Of particular interest, from our point of view, is the study of biogenic amines in lung tissue of lung cancer patients who have undergone COVID-19.

It has been shown that histamine and the histamine receptor signaling pathway can play a crucial role in the penetration of the virus into endothelial cells. SARS-CoV-2 causes an inflammatory reaction in infected patients [11]. The authors believe that a local increase in histamine levels, for example, in the lungs, brain or heart, can change the penetration of the virus into cells directly in the body. Wu M. L. et al. (2022) showed that mast cell degranulation caused by spike protein initiates inflammation of the alveolar epithelium to destroy the barrier, and suggested using antihistamines not for their intended purpose, but as mast cell stabilizers to block degranulation and, consequently, suppress inflammation and prevent lung damage [16]. High levels of histamine and histamine receptors, including H1R~H4R, were found in various types of tumor cells and cells of the tumor microenvironment, which suggests their involvement in tumor progression [17].

Research by Fabre A. with co-authors (2008) [18] showed the involvement of serotonin in the pathophysiology of bleomycin-induced pulmonary fibrosis in mice and identified it as a potential therapeutic target for fibrotic lung diseases. Serotonin (5-hydroxytryptamine; 5-HT) is a vasoactive peptide synthesized from tryptophan by enterochromaffin cells of the intestine and endothelial cells. There is a very low level of circulating free serotonin in the blood, since most of the serotonin is concentrated in platelets. Under physiological conditions, the lungs

are exposed to low levels of circulating serotonin. In pathological conditions, the release of serotonin stored by platelets and endothelial cells can increase the concentration of serotonin both locally and in the bloodstream. It has been shown that 5-HT2A and 5-HT2B receptor subtypes play the most important role in the lungs, where serotonin is involved in the control of vaso- and bronchoreactivity [18]. Studies have shown the potential stimulating effect of serotonin on proliferation, invasion, dissemination of cancer cells and tumor angiogenesis. Although the underlying mechanism is complex, it is assumed that serotonin levels in the tumor and its interaction with specific receptor subtypes are associated with disease progression [19].

The role of norepinephrine in malignant growth is associated with cancer cell survival, proliferation, resistance to apoptosis, invasion, metastasis, angiogenesis and stromal compartments in the tumor microenvironment [20]. Norepinephrine can inhibit oxidative phosphorylation and induce angiogenic switching, stimulating the metabolism of endothelial cells in a direction that promotes cancer progression [21]. There are several mechanisms underlying the role of norepinephrine in the development of tumors. Activation of β2-adrenoreceptors promotes tumor growth and angiogenesis by increasing the expression of vascular endothelial growth factor (VEGF), metalloproteases MMP2 and MMP9, which additionally potentiate angiogenic and metastatic processes in adrenal cancer, lung cancer, and breast cancer. These effects are largely mediated by a β-adrenergic increase in cyclic adenosine monophosphate (cAMP) levels and subsequent activation of protein kinase A, which performs the corresponding functional regulation by phosphorylation of downstream targets, such as protein binding the cAMP response element, nuclear factor kappa B and activator protein 1 [22].

Dopamine is a biological precursor for the synthesis of norepinephrine. In addition to the common angiotensin-converting enzyme receptor 2, new coronaviruses may use other alternative pathways. The first putative receptor or coreceptor is dopamine. Dopamine is known to reduce SARS-CoV-2 replication *in vitro* by suppressing its D2 receptors and enhancing type I interferons [23]. Recent evidence suggests that SARS-CoV-2 interferes with immune responses through dopamine-related mechanisms [24]. However, dopamine can suppress the immune response during infection, thereby increasing the life cycle of SARS-CoV-2. Elevated dopamine levels reduce oxygen levels, especially when considering the "happy" hypoxemia associated with COVID-19 [23]. This happens because dopamine has a known ability to dull the ventilation response of the basal carotid arteries of a person to hypoxia. However, to date, no studies have examined the potential role of dopamine and dopaminergic receptors in COVID-19 infection.

As for the role of dopamine in cancer, the available information is quite contradictory. Some epidemiological and molecular biological studies have shown that dopamine has different effects on different types of cancer and even on cancer of the same localization [22]. These results mean that an imbalance in the dopaminergic system may be associated with the development of a malignant tumor.

Purpose of the study was to study the content of biogenic amines – serotonin and its metabolite 5-HIAA, dopamine, norepinephrine and histamine in lung tissues in patients with lung cancer who had suffered COVID-19 of varying severity.

PATIENTS AND METHODS

The material for the study was samples of lung tissue outside the tumor growth zone, tumor tissue and its perifocal zone obtained as a result of open biopsy during radical operations in patients with morphologically verified non-small cell lung cancer (NSCLC) of stage I–IIIA ($cT_{1-3}N_xM_0$). The study included 60 male and female patients in a 1:1 ratio who underwent a standard X-ray examination before surgery to exclude metastatic disease: computed tomography (CT) of the chest and abdominal cavity, as well as brain imaging (CT or MRI); part of the patients underwent 1-F-FDG PET-CT, but this study was optional. Osteoscintigraphy was performed according to clinical indications. The clinical stage of NSCLC was established using the TNM lung Cancer classification Union for International Cancer Control / American Joint Committee on Cancer classification system, version 8.

Patients who had suffered SARS-CoV-2 infection 3–6 months ago, aged 18 years and older with morphologically verified NSCLC, who were scheduled for radical surgical intervention in the volume of lobectomy, bilobectomy or pneumonectomy with mediastinal lymph dissection, were considered suitable.

The initial status of the patients should have been 0–2 points on the ECOG scale, the volume of forced exhalation in 1 second more than 1.5 liters or more than 70 % of the required and normal indicators of standard laboratory tests, including a general blood test, biochemical serum analysis, coagulogram, indicating the absence of functional disorders of organs and systems. The study did not include patients who had undergone neoadjuvant chemotherapy, with synchronous contralateral lung cancer, with malignant neoplasms of other localizations, except basal cell skin cancer, in the past or current history and with recent less than 6 months. severe cardiac, pulmonary or inflammatory diseases, except COVID-19, as well as patients with diabetes mellitus.

Upon hospitalization, all patients had a negative PCR test for SARS-CoV-2 from the nasopharynx. Based on anamnestic data collected using a special questionnaire, depending on the severity of the clinical course of COVID-19, the main and control groups were formed. The main group included 30 patients with NSCLC (15 men and 15 women) who had suffered COVID-19 in severe and moderate form that required hospitalization, the control group similarly consisted of 30 patients with lung cancer in whom SARS-CoV-2 infection was asymptomatic or mild. The average age of the patients was 59.11 ± 2.9 years, there were no significant differences between the compared groups in terms of anthropometric and clinical indicators.

The study participants gave written informed consent to medical intervention, surgery, processing of personal data and collection of biological material in accordance with the Helsinki Declaration. The study was approved by the Ethics Council of the NMRC for Oncology of the Ministry of Health of Russia, Rostov-on-Don (Protocol No. 6 of 01/17/2022).

Quantitative assessment of the content of dopamine, norepinephrine, serotonin, 5-hydroxyindolacetic acid (5-HIAA) and histamine was performed by the ELISA method using standard kits (IBL; Germany).

Statistical analysis was carried out using the Statistica 10 program. The normality of the distribution was assessed using Kolmogorov-Smirnov methods, differences between groups were determined using the Student's t-test or the Mann-Whitney U-test, depending on the normality of the distribution. The value of $p < 0.05$ was considered as an indicator of statistical significance.

RESEARCH RESULTS

It was found that in intact tissue (resection line) of men and women of the main group, the level of dopamine and norepinephrine was lower than in the corresponding samples of patients of the control group: in men on average 1.3 times, in women on average 1.4 times. Since dopamine is a precursor to norepinephrine, we found it interesting to study the ratio of dopamine and norepinephrine. The ratio of dopamine to norepinephrine (D/NA) had no significant differences, both between groups and depending on gender (Table 1). The histamine level in the patients of the main group had no significant differences from the values in the control group, however, in both groups the indicator was higher in men than in women: in the control group – 2.5 times, in the main group – 2.3 times. The serotonin content was 1.5 times higher in patients of the control group on average, regardless of gender. Significant differences were found in intact tissue and in the content of the serotonin-5-HIAA metabolite. The values of this indicator were 4.2 times and 2.8 times higher in men and women of the control group, respectively. At the same time, gender differences are noted: in men, the indicator was 1.9 times higher than in women in the control group, in the main group – 1.3 times. The ratio of serotonin/5-HIAA (5-HT/5-HIAA) reflects the process of synthesis/decay of this biogenic amine. It turned out that in patients of the main group, this indicator was higher than in the control group: in men – 2.7 times, in women – 1.9 times. At the same time, there were gender differences: in the control group, the indicator for women exceeded the values for men by 1.5 times. In the main group, 5-HT/5-HIAA was the same for women and men.

In the tumor tissue of men and women of the main group, the level of dopamine had no significant differences from the corresponding samples of patients of the control group, only in the main group the level of D in the tumor was 1.5 times higher in women, whereas in the control group no sex differences were detected.

At the same time, relative to the indicators in intact tissue, the level of dopamine in the tumor tissue of male and female patients of the control group was reduced by 1.3 times and 1.4 times, while the values in the tumor tissue of patients of the main group had no significant differences. The content of norepinephrine in the tumor tissue of patients in the control group,

Table 1. The content of biogenic amines and their ratios in the lung tissues of patients with NSCLC, depending on the severity of COVID-19

Groups	Sex	D ng/g t	NA ng/g t	5-HT ng/g t	5-HIAA ng/g t	Histamine ng/g t	D/NA	5HT / 5HIAA
Resection line tissue								
Control	M	72.8 ± 7.4	60.1 ± 6.5	2.0 ± 0.19	0.54 ± 0.06	1572.9 ± 159.4	1.2 ± 0.02	3.8 ± 0.14
	F	85.7 ± 7.9	74.1 ± 9.1	1.6 ± 0.17	0.28 ± 0.02 $p^2 = 0.0001$	634.9 ± 71.9 $p^2 = 0.0000$	1.22 ± 0.05	5.6 ± 0.2 $p^2 = 0.0000$
Main	M	55.6 ± 3.9 $p^1 = 0.0495$	44.7 ± 3.4 $p^1 = 0.0455$	1.3 ± 0.14 $p^1 = 0.0062$	0.13 ± 0.01 $p^1 = 0.0000$	1437.3 ± 135.8	1.26 ± 0.04	9.9 ± 0.45 $p^1 = 0.0000$
	F	66.2 ± 5.1 $p^1 = 0.0477$	51.2 ± 6.1 $p^1 = 0.0462$	1.1 ± 0.12 $p^1 = 0.0234$	0.1 ± 0.008 $p^1 = 0.0000$ $p^2 = 0.0374$	628.4 ± 71.4 $p^2 = 0.0000$	1.38 ± 0.07	10.8 ± 0.61 $p^1 = 0.0000$
Tumor tissue								
Control	M	54.2 ± 3.97 $p^3 = 0.0350$	113.6 ± 5.47 $p^3 = 0.0000$	3.3 ± 0.22	0.25 ± 0.02	2026.9 ± 129.4 $p^3 = 0.0353$	0.47 ± 0.02 $p^3 = 0.0000$	13.35 ± 0.21 $p^3 = 0.0000$
	F	60.1 ± 3.2 $p^3 = 0.0138$	118.8 ± 6.1 $p^3 = 0.0003$	3.8 ± 0.39	0.27 ± 0.04	1992.7 ± 210.6 $p^3 = 0.0000$ $p^4 = 0.0014$	0.51 ± 0.01 $p^3 = 0.0000$	14.5 ± 0.79 $p^3 = 0.0000$
Main	M	45.3 ± 4.9	37.5 ± 3.8 $p^1 = 0.0000$	2.1 ± 0.23 $p^1 = 0.0008$	0.2 ± 0.02	1901.7 ± 155.9 $p^3 = 0.0327$	1.2 ± 0.02 $p^1 = 0.0000$	10.6 ± 0.19
	F	67.8 ± 7.4 $p^2 = 0.0170$	35.6 ± 3.9 $p^1 = 0.0000$	2.8 ± 0.26 $p^1 = 0.0441$	0.2 ± 0.02	1571.1 ± 165.0 $p^3 = 0.0000$ $p^4 = 0.0060$	1.92 ± 0.04 $p^1 = 0.0000$ $p^2 = 0.0000$ $p^3 = 0.0000$	14.3 ± 0.77
Periodical zone tissue								
Control	M	71.8 ± 7.8	64.5 ± 7.2	1.7 ± 0.18	0.5 ± 0.07	1710.1 ± 190.7	1.1 ± 0.01	3.7 ± 0.24
	F	77.8 ± 8.4	67.1 ± 7.36	1.8 ± 0.19	0.3 ± 0.04 $p^2 = 0.0220$	1135.3 ± 121.2 $p^3 = 0.0013$	1.2 ± 0.03	6.2 ± 0.29 $p^2 = 0.0000$
Main	M	55.5 ± 5.6	51.5 ± 5.7	1.4 ± 0.16	0.18 ± 0.02 $p^1 = 0.0002$ $p^3 = 0.0392$	1473.9 ± 161.2	1.1 ± 0.06	7.8 ± 0.17
	F	63.3 ± 6.4	43.7 ± 4.5	1.5 ± 0.18	0.17 ± 0.02 $p^1 = 0.0036$ $p^3 = 0.0077$	942.9 ± 103.2 $p^3 = 0.0182$	1.4 ± 0.03	8.8 ± 0.29

Note: statistically significant in relation to: [1] – to the corresponding indicator in the control group; [2] – to the indicator in men in the corresponding group; [3] – to the corresponding indicator in the tissue of the resection line; [4] to the corresponding indicator in the tissue of the perifocal zone; g t – grams of tissue. D – dopamine, NA – norepinephrine, 5NT – serotonin, 5HIAA – 5-hydroxyindoleacetic acid.

regardless of gender, was 3.2 times higher than the level of biogenic amine in the tumor tissue of the main group on average. At the same time, relative to intact tissue, the indicators were increased in men and women of the control group by 1.9 times and 1.6 times, respectively, and in the main group had no significant differences. The ratio is D/NA the tumor tissue of patients of the control group was lower than in patients of the main group: in men by 2.4 times, in women by 3.9 times. Gender differences were noted only in the main group of patients: in women, the indicator was 1.6 times higher. With respect to intact tissue, the coefficient is D/NA in the tumor tissue of patients in the control group, regardless of gender, was reduced by an average of 2.4 times.

In the tumor tissue of the main group, relative to the corresponding intact tissue, the level of D/NA was increased in women by 1.5 times, and had no significant differences in men. The histamine content in the tumor tissue of patients of the control and main groups did not differ either between the groups or depending on gender, but was increased relative to the values in the corresponding intact tissue: in men and women of the control group by 1.3 times and 3.1 times, respectively, in men and women of the main group – by 1.3 times and 2.4 three times, respectively. The level of serotonin in the tumor tissue of patients in the control group was 1.4 times higher than in the main group, regardless of gender, on average, and the level of its metabolite 5-HIAA K had no significant differences. The 5-HT/5-HIAA coefficient in the tumor tissue did not differ between the groups and did not differ by gender, but in the control group there were differences with the indicators in the corresponding intact tissue: in men, the indicator was 3.6 times higher, in women – 2.5 times.

In the tissue of the perifocal zone, the content of dopamine, norepinephrine and their ratios in all the studied samples had no significant differences from the indicators in the corresponding intact tissue and tumor. There were also no significant differences in the content of serotonin in the samples of the perifocal zone and the corresponding intact tissue, but at the same time, the level of 5NT was lower than in the tumor itself by an average of 1.5–2 times. In the perifocal zone, the level of 5-HIAA did not differ from the indicators of the corresponding intact tissue in patients of the control group, whereas there were differences in the samples of patients of the main group. So in men, the level of the metabolite was 1.4 times higher, and in women – 1.7 times. In this regard, the ratio of 5-HT/5-HIAA in the tissue of the perifocal zone in patients of the control group had no significant differences from the values in the intact tissue, maintaining the same sex differences – 1.8 times higher in women. In the tissue of the perifocal zone of the tumor of patients of the main group, the ratio of 5-HT/5-HIAA also had no differences with the corresponding intact tissue and tumor tissue. The histamine level in the tissue of the perifocal zone of the tumor in men of both groups had no significant differences from the values in the corresponding intact tissue, and in women of both groups, the level of this diamine occupied an intermediate position between the indicators in the corresponding intact tissue and tumor tissue. Thus, in the tissue of the perifocal zone of the tumor of women of the control and main groups, the histamine level was 1.8 times and 1.5 times higher, respectively, than in the intact lung tissue, but 1.8 times and 1.6 times lower, respectively, than in the tumor.

Thus, it was found that in all the studied lung tissue samples of men and women of the main group, compared with the control group, there is an insufficiency of catecholamines without violating their ratio and a change in serotonin metabolism to ensure its level. Obviously, changes in the biogenic status of lung tissues may be associated with the severity of the SARS-CoV-2 infection.

DISCUSSION

Neurotransmitters, which include biogenic amines, are usually considered as substances secreted by nerves that mediate the stimulating or inhibitory functions of neurons by binding to the corresponding receptors. In recent decades, many new discoveries have appeared explaining the regulatory role of neurotransmitters in the physiological and pathological functions of tissues and organs. It is noteworthy that new data suggest that cancer cells use a signaling pathway initiated by neurotransmitters to activate uncontrolled proliferation and dissemination [22]. In addition, neurotransmitters can affect immune cells and endothelial cells in the tumor microenvironment, regulating tissue homeostasis, influencing various tumor phenotypes and contributing to tumor progression [22; 24; 25]. Neurotransmitters can affect cancer

cells and immune cells in an autocrine/paracrine way. Similar to the processes of neoangiogenesis and lymphangiogenesis, more and more evidence points to the possibility of the formation of new nerve endings in tumors, a phenomenon called neoneurogenesis [25]. Neurotransmitters of nerve fibers released in the tumor microenvironment activate tumor cells by binding specific neurotransmitter receptors. This process has further expanded our knowledge of the complex network of neurotransmitters associated with tumor progression. In addition, immune cells and endothelial cells infiltrated in the tumor microenvironment also express various neurotransmitter receptors and react with neurotransmitters, which is known to have a strong effect on the outcome of cancer in humans [26].

In this study, it was shown that in lung tissue unaffected by the malignant process (resection line), there were gender differences in the level of histamine, regardless of the severity of COVID-19: in men, its content was more than twice as high as in women [27]. This fact may be associated, on the one hand, with inflammatory changes in lung tissue in men, on the other, with an increased possibility of virus penetration into cells, and possibly with hormonal security of the tissue. In the tumor tissue, regardless of gender and infection, the histamine level was higher than in the corresponding conditionally intact tissue, which indicates the likelihood of its participation in tumor progression [17]. The histamine content in the tissue of the perifocal zone of the tumor occupied an intermediate position between the tumor tissue and the resection line, which indicates the involvement of this zone in the process of carcinogenesis.

Non-small cell lung cancer (NSCLC) is the most common form of lung cancer and is characterized by a chronic inflammatory process, which is associated with high infiltration of mast cells and a decrease in patient survival [28]. It is known that high levels of histamine and histamine receptors in a wide range of different types of cancer, including lung cancer, indicate their involvement in the complex biology of cancer [29]. In addition, histamine has been shown to stimulate various events related to carcinogenesis, such as cell invasion, migration, and angiogenesis, demonstrating its crucial role in cancer progression [17].

5-HT is known to regulate epithelial homeostasis of the breast, lungs, pancreas, liver and prostate. A violation of the regulation of 5-HT signaling is often observed in epithelial tumors [19]. We have shown that the absolute level of serotonin, as well as the ratio of 5-HT/5-HIAA in the tumor tissue in the control group were significantly higher than in the conditionally intact lung tissue and tissue of the perifocal zone of the tumor and had no fundamental differences depending on gender.

The available data clarified the biology of the tumor from the positions of 5-HT. It was found that 5-HT promotes cell proliferation in cancer through various 5-HT receptors [30]. Jiang S. H. and co-authors (2017) demonstrated that human pancreatic cancer tissues have elevated levels of 5-HT, and pancreatic cancer cells increase the expression of its receptor, HTR2B. This increase promotes tumor glycolysis under metabolic stress and promotes the growth of pancreatic cancer [22].

However, we drew attention to the fact that in patients of the main group, the level of serotonin and the ratio of 5-HT/5-HIAA in the tumor tissue and its perifocal zone had no fundamental differences from the indicators in the control group of patients, and the ratio of 5-HT/5-HIAA in intact tissue along the resection line was significantly different from the indicators in the control group and was similar to the indicators in the tumor tissue of patients in the main group. This could not be explained in any way from the standpoint of the participation of serotonin in tumor progression, since in the tissue of the corresponding perifocal zone, the indicators were significantly lower, as well as in patients of the control group. We considered that this was due to the severity of COVID-19, namely, the ability of serotonin to influence the formation of fibrosis in the lungs. Moreover, in the intact tissue of patients of the main group who had suffered severe and moderate infection, the relative level of serotonin was increased not due to its de novo synthesis, but due to a sharp drop in its decay.

The results obtained by Petrić M. with co-authors (2022) indicate that serotonin may play a profibrotic role in lung tissue, especially due to the fact that half of patients with low lung capacity had interstitial lung diseases [31]. In mouse models, data were obtained that serotonin is involved in the pathogenesis of pulmonary fibrosis through 5-HT/Akt signaling pathways and enhanced TGF-β1-induced collagen synthesis [32]. A Swedish group of authors identified the serotonin receptor as a therapeutic target for the

fibrosing phenotype of interstitial lung diseases [33]. At the same time, there is a study showing a violation of the regulation of tryptophan metabolism, characterized by a decrease in serotonin production in combination with the accumulation of quinoline in patients with COVID-19 during the acute phase of infection, especially in patients with the most unfavorable outcomes [34].

Perhaps in situations where available serotonin levels are reduced, inhibition of its local metabolism may have a beneficial effect, helping to maintain adequate levels of serotonin signaling.

There are several mechanisms underlying the role of adrenaline and norepinephrine in the development of tumors. Norepinephrine can stimulate the metabolism of endothelial cells in the direction of inhibition of oxidative phosphorylation and induction of angiogenic switching, which contributes to the progression of cancer [21].

In our study, it was shown that in the tumor tissue of patients in the control group, the level of norepinephrine was significantly higher than in the corresponding intact tissue and tissue of the perifocal zone of the tumor, which is consistent with the literature data. No gender differences were noted. The other was observed in lung tissues of patients of the main group. In the tissue of the resection line of these patients, the level of norepinephrine was significantly lower than in the intact tissue of men and women with lung cancer of the control group. The same pattern was found in the tumor tissue and in the tissue of its perifocal zone. At the same time, there were no differences in indicators between all samples, i.e. the level of norepinephrine was approximately the same, and significantly lower than in patients of the control group. Explanations for this fact were difficult to find. Then we turned to the content of dopamine in the lung tissues of patients with NSCLC, since it is known that dopamine is a precursor of norepinephrine.

It is known that hormones such as glucagon, adrenaline or norepinephrine trigger an intracellular signaling cascade that triggers the activation of protein kinase A. Dopamine weakens the activation of protein kinase A, which is initiated by lowering the cellular level of cAMP. Protein kinase A activates the NF-kB pathway by phosphorylation of p65 by serine 276, which promotes oncogenesis of NSCLC in mice and significantly correlates with progressive stages of TNM and poor prognosis in patients with NSCLC [22].

We found that the levels of dopamine, as well as norepinephrine in the intact tissue of men and women of the main group were significantly lower than in the corresponding samples of patients of the control group, possibly due to a violation of tyrosine metabolism. The metabolism of amino acids in the tumor microenvironment plays a key role in the development and progression of the tumor. Tumor cells often consume exclusively local nutrients, such as amino acids, for their survival and compete for them with other surrounding cells, such as antitumor immune cells [35]. In the tumor tissue of patients of both sexes of the control group, the level of dopamine was reduced due to increased norepinephrine formation. Not equivalent changes were found in tumor tissue in men and women of the main group. Thus, the levels of dopamine and norepinephrine in the tumor tissue of men were reduced almost as well as in intact tissue, and in women the content of norepinephrine was kept almost at the level of values in intact tissue and tissue of the perifocal zone, dopamine was significantly higher, as well as in the tissue of the perifocal zone.

It is known that dopamine is involved in countering the carcinogenic action of the adrenergic system. The antitumor effects of dopamine can manifest themselves in the regulation of various immunocompetent cells in the tumor microenvironment. Dopamine is able to inhibit the function of Gr-1 + CD115+ suppressor cells of myeloid origin through D1-like receptors and enhance antitumor immunity [22]. Recent intriguing evidence suggests that SARS-CoV-2 may interfere with immune responses through dopamine-related mechanisms. On the one hand, it has been suggested that dopamine receptors are used by the virus to improve both its penetration and the life cycle inside cells; on the other hand, it has been documented that SARS-CoV-2 suppresses L-DOPA decarboxylase, an enzyme that limits the rate of conversion of L-DOPA to dopamine, which, obviously affects the content of normadrenaline [36].

CONCLUSION

Obviously, changes in the levels of dopamine, norepinephrine and serotonin in lung tissues may be associated with the severity of SARS-CoV-2 infection

and should be reflected in the course of lung cancer. The influence of biogenic amines on tumor development and progression includes their direct role in tumor growth and metastasis not only through cancer cells and stromal cells, but also through endothelial cells and immune cells, contributing to angiogenesis, lymphangiogenesis and inflammatory response. The transferred COVID-19 contributes its modifying effect on the biogenic status in the lung in patients of the main group, which can lead to a more severe course of the malignant process and a change in the response to standard antitumor therapy.

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ORIGINAL ARTICLE

PARAMETERS OF FREE RADICAL OXIDATION AND ANTIOXIDANT DEFENSE IN PATIENTS WITH CERVICAL CANCER BEFORE AND AFTER RADICAL SURGICAL TREATMENT

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ABSTRACT

Purpose of the study. To analyse free radical oxidation and antioxidant defense in patients diagnosed with early cervical cancer (CC) before and after radical surgical treatment.
Patients and methods. Levels of diene conjugates, malondialdehyde (MDA), superoxide dismutase (SOD), catalase, glutathione, glutathione-dependent enzymes, vitamins A and E were determined in 74 women under the age of 45 (48 patients those who were at the stage of surgical treatment with a diagnosis of CC at the National Medical Research Center of Oncology in the period 2017–2020 and 26 healthy women).
Results. Patients with early CC showed significant changes in the intensity of lipid peroxidation processes and in antioxidant defense: elevate levels of MDA and diene conjugates, initial decline in the activity of SOD and catalase, low levels of vitamins A and E. These results complete the understanding of the processes occurring in the body of an oncological patient at the initial stage of tumor formation, which does not yet have an obvious clinical manifestation. After total removal of the ovaries, most of the indicators characterizing the enzymatic link of the antioxidant system tend to normalize, while the violation of the content of vitamins E and A (related to the non-enzymatic link of the antioxidant system) worsens.
Conclusions. Desynchronization of free radical oxidation processes with multidirectional changes in oxidation and antioxidation in patients with early CC at the stage of radical surgical treatment should be considered from the position of hormone-reducing surgery and a resulting complex of changes in the organs and systems of women with cancer.

Keywords: cervical cancer, lipid peroxidation, catalase, malondialdehyde, superoxide dismutase, glutathione

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Compliance with ethical standards: the ethical principles presented by the World Medical Association Declaration of Helsinki, 1964, ed. 2013 were observed in the study. The study was approved by the ethics committee of the National Medical Research Centre for Oncology (extract from the protocol of the meeting No. 5 dated 09/14/2019). Informed consent was received from all participants of the study.

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3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

ПОКАЗАТЕЛИ СВОБОДНОРАДИКАЛЬНОГО ОКИСЛЕНИЯ И АНТИОКСИДАНТНОЙ ЗАЩИТЫ У ПАЦИЕНТОК С ДИАГНОЗОМ «РАК ШЕЙКИ МАТКИ» ДО И ПОСЛЕ ПРОВЕДЕНИЯ РАДИКАЛЬНОГО ХИРУРГИЧЕСКОГО ЛЕЧЕНИЯ

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РЕЗЮМЕ

Цель исследования. Проанализировать состояние свободнорадикального окисления и антиоксидантной защиты у пациенток с диагнозом «рак шейки матки» (РШМ) ранних стадий до и после проведения радикального хирургического лечения.

Пациенты и методы. Исследовали уровень диеновых конъюгатов, малонового диальдегида (МДА), супероксиддисмутазы (СОД), каталазы, глутатиона, глутатионзависимых ферментов, содержание витамина А и Е у 74 пациенток в возрастной категории до 45 лет (48 больных, находившихся на этапе хирургического лечения с диагнозом РШМ в ФГБУ «НМИЦ онкологии» Минздрава России в период 2017–2020 гг. и 26 здоровых женщин).

Результаты. У больных РШМ на начальных стадиях заболевания выявлены существенные изменения в интенсификации реакций перекисного окисления липидов и в антиоксидантной системе: повышенный уровень МДА и диеновых конъюгатов, исходное снижение активности СОД и каталазы, низкие показатели витамина Е и А. Данные результаты дополняют представления о процессах, происходящих в организме онкологического больного на начальном этапе формирования опухоли, которая ещё не имеет явного клинического проявления. После тотального удаления яичников большинство показателей, характеризующих ферментативное звено антиоксидантной системы, проявляют тенденцию к нормализации, в то время как нарушение содержания витаминов Е и А (относящихся к неферментативному звену антиоксидантной системы) усугубляется.

Заключение. Десинхронизацию процессов свободнорадикального окисления с разнонаправленными изменениями процессов окисления и антиокисления, у больных РШМ ранних стадий на этапе радикального хирургического лечения следует рассматривать с позиции гормонредуцирующей операции и связанного с ней сложного комплекса изменений в органах и системах женщин с онкологической патологией.

Ключевые слова: рак шейки матки, перекисное окисление липидов, каталаза, малоновый диальдегид, суперокиддисмутаза, глутатион

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Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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RELEVANCE

The incidence of cervical cancer ranks 2nd (5.3 %) in the structure of oncogenital pathology in Russia, after uterine cancer (8.0 %) and 5th-6th in the structure of the entire oncopathology of the female population [1–3]. The tendency to increase the frequency of developing CC in women of reproductive age is alarming [4; 5]. The highest mortality rates from this pathology are recorded in the age group of 15–39 years (21.6 %) [6]. The increase in morbidity and mortality rates among young women diagnosed with CC [7] contributes to the active search for diagnostic and therapeutic directions with the effective integration of new scientific research. Modern trends in surgical treatment of oncogynecological patients are aimed at carrying out organ-preserving operations. According to the practical recommendations for the treatment of CC of localized stages, i.e. IA, IB1 and IIA1-2 from 2021, patients are shown to perform modified uterine extirpation (classification according to Piver III). Women diagnosed with CC under 45 years of age may retain ovarian function [8]. However, in some cases, if patients are not interested in preserving reproductive function, as well as in compliance with the principles of radical treatment, total removal of the affected organ and the most likely metastatic niches is used. Thus, the risk of implantation metastasis in ovarian cancer, the age of the patient and concomitant hormonal pathology determine the scope of the operation, i.e. extirpation of the uterus with appendages and expanded lymphadenectomy [9]. Forced and irreversible suppression of the ovaries leads to the formation of a pathological complex of disorders in the patient's body, which is due not only to the carcinolytic effects of the tumor process, but also to hypoestrogenia with a detailed metabolic picture of an artificially created menopausal state [10]. Postovariectomy syndrome (POES) manifests itself in the form of disorders of homeostasis, metabolic processes, neuroendocrine regulation and psychoemotional status, and is also characterized by changes in the state of pro- and antioxidant systems [9]. In addition, it is known that the growth and development of a malignant tumor, as well as the effect and negative manifestations of antitumor therapy largely depend on systemic disorders in the mechanisms of regulation of redox processes. During the formation of malignant transformation and the progression of the process, pathogenetically significant changes in the functional systems of the body of an oncological patient are generally recognized. There are results of studies where the role of oxidative stress and imbalance of redox processes in tumor growth is determined [11]. The activation of free radical oxidation (FRO) processes with the generation of activated oxygen metabolites in the regulation of cellular programs is proven [12]. There are studies that show significant changes in the level of the main antioxidant enzymes and vitamins A and E in patients with HPV (human papillomavirus) – associated forms of CC [13].

In order to improve the diagnosis and identify the patterns of development of CC, it is necessary to further study the pathophysiological changes occurring in the body of women of reproductive age at the early stages of the process and as a result of antitumor treatment. The results of research by domestic and foreign authors indicate that in the process of formation and progression of a malignant tumor, there is an increased activity of SRO with depletion of factors of the antioxidant system of the body of an oncological patient [14]. A number of key indicators of lipid peroxidation (LPO) and the activity of antioxidant protection of the blood of an oncological patient are informative in assessing the extent of the prevalence and progression of the tumor process. Long-term experimental and clinical studies conducted on the basis of the National Medical Research Center of Oncology allow us to characterize the state of redox reactions in most of the studied oncogynecological processes, which undoubtedly acquires practical significance [15; 16]. Thus, the basis of the method of treatment of patients with a primarily unresectable form of CC, including a combination of neoadjuvant chemotherapy and plasmapheresis procedure, were revealed violations among the indicators of free radical processes in endogenous intoxication in cancer patients at the stages of therapy [17]. At the same time, questions remain open about the dynamics of the indicators of LPO and the antioxidant system in patients with early-stage CC, which may be prognostically relevant in assessing the progression of the disease and the effectiveness of the antitumor treatment.

The aim of the study: to conduct a comparative analysis of the activity indicators of free radical oxidation and antioxidant protection processes in the blood of reproductive-age patients diagnosed with early-stage cervical cancer before and after radical surgical treatment.

PATIENTS AND METHODS

The blood parameters characterizing the state of activity of oxidative processes and antioxidant enzymes in 74 patients of reproductive age (up to 45 years) were studied. Of these, the main group consisted of 48 patients diagnosed with CC, hospitalized for complex treatment in the Department of Oncogynecology of the NMRC for Oncology of the Ministry of Health of Russia, and a comparison group of 26 women without oncopathology. The criterion for inclusion in the groups of this study was the absence of autoimmune and endocrine pathology, obesity, exacerbation of chronic pathology, as well as constant medication intake. The study was conducted with the approval of the Ethical Committee of the institution and with the voluntary consent of patients to use biological material with the processing of their personal data for scientific purposes. The main group is represented by patients diagnosed with CC, who underwent modified extirpation of the uterus with appendages (classification according to Piver III) as the first stage of antitumor treatment. According to the clinical examination, the distribution of the stage of oncological disease (classification TNM, 2019) is presented: $T_{1a2}N_0M_0$ in 5 patients (10.4 %), $T_{1b1}N_0M_0$ in 7 patients (14.7 %), $T_{1b2}N_0M_0$ in 36 patients (74.9 %). Morphological characteristics of the tumor: squamous cell carcinoma was detected in 40 patients (83.2 %), adenocarcinoma was diagnosed in 8 patients (16.8 %). A moderate degree of differentiation was detected in 27 patients (56.2 %). 97.4 % of patients had a positive HPV test of high carcinogenic risk. All patients of the group, in the early postoperative period, had signs of POES to one degree or another. The median age in the main group was 37 years, the average age was 41.6 ± 1.7, the range was 26–45 years. The body mass index (BMI) in the group was 23.8 ± 0.16 kg/m^2. Stages of the study: before surgical treatment and the 2nd day of the postoperative period. The comparison group consisted of 26 relatively healthy women, employees of the center, comparable to the group of patients with CC by age and BMI. The median age is 39 years, the average age is 43.2 ± 1.5, the range is 28–45 years. The BMI in the group without oncopathology was 23.3 ± 0.4 kg/m^2.

The intensity of LPO in erythrocytes and blood plasma was determined by the indicators of diene conjugates [18] and malondialdehyde (MDA) [19]. To judge the state of the antioxidant system, the activity of superoxide dismutase, catalase, glutathione-dependent enzymes, the level of glutathione [20], the content of vitamin A and E were determined by Chernyauskene R. Ch. method[21]. To determine these indicators, venous blood sampling from patients was carried out from the ulnar vein, in the morning, on an empty stomach.

Statistical results were processed using Statistika 10 programs according to the Student's criterion for two independent samples and the nonparametric Wilcoxon-Mann-Whitney criterion, the differences were considered statistically significant at $p < 0.05$. The samples were checked for compliance with the normal distribution according to the Kolmogorov-Smirnov criterion and the Shapiro-Wilk W-criterion. Since in most cases the distribution was close to normal, the results are presented in the form of $M \pm m$, where M is the sample mean, m is the error of the mean, the median (*Me*), which in all groups practically did not differ from M, and the interquartile spread in the form of counting the lower and upper quartiles: (Q25 and Q75).

RESEARCH RESULTS

The most informative integral indicators for assessing the intensity of LPO processes in the blood of patients are the content of primary products of diene conjugates and malondialdehyde (Table 1).

The status of antioxidant protection in patients with CC at the surgical stage of treatment was determined by several indicators: activity of catalase and superoxide dismutase enzymes, the level of reduced glutathione and glutathione-dependent enzymes (glutathione peroxidase, glutathione transferase, glutathione reductase). The data is shown in table 2.

Vitamin A and E levels in blood samples of CC patients before and after the surgery was the subject of special interest. The obtained data is brought in the table 3.

DISCUSSION

Malonic dialdehyde (MDA) is a secondary molecular product of LPO, formed in the body due to the reactions of ROS and polyunsaturated fatty acids. According to modern concepts, MDA is considered as a biological marker of changes in the system of free radical lipid oxidation [22]. When studying the initial activity of SEX processes in patients with a diagnosis of CC in comparison with healthy women, a significant increase in MDA was recorded, in blood plasma by 75.9 % (p = 0.002368), in erythrocytes by 59.7 % (p = 0.0108), the results are shown in Table 1.

According to our study, it was found that in patients with CC, the level of primary LPO products, which include diene conjugates, was significantly increased before the surgical stage of antitumor treatment. This indicator had significant differences with the group without oncopathology. So, in blood plasma, the indicator exceeded the values by 4.4 times (p = 0.000011), and in erythrocytes by an average of 6.6 times (p = 0.000038). However, a significant variation in the level of diene conjugates in erythrocytes was detected in the group of patients with CC, no changes were recorded in 42 % of cases, while a 14-fold increase in indicators was observed in 58 % of cases. After surgery, the level of diene conjugates in the group of patients with CC had a decrease of 5.3 times relative to the baseline level in erythrocytes, plasma, also recorded a decrease relative to the group of healthy and remaining 3.6 times higher than normal (p = 0.0033) (Table 1).

It is known that the main protective role under the influence of oxidative stress belongs to the non-enzymatic link of antioxidants (glutathione, vitamins A, E) and enzymatic antioxidants (superoxide dismutase (SOD), glutathione peroxidase, catalase) [23]. The results of the functioning of non-enzymatic and enzymatic antioxidant systems play an important role in the regulation of carcinogenesis, including in the processes that stimulate tumor progression, as well as the formation of its resistance to therapy [14].

Superoxide dismutase refers to the enzymes of the first line of defense of the antioxidant system.

Table 1. LPO indices in the blood plasma and erythrocytes in healthy women and CC patients before and after the surgery

Index *M ± m* Me (Q25; Q75)	Study group		
	Healthy women, *n* = 26	CC patients before treatment, *n* = 48	CC patients after surgery, *n* = 48
MDA in blood plasma (nM/ml)	7.075 ± 0.638 6.937 (4.9; 8.4)	12.476 ± 1.21 11.1 (4.245; 19.85) *p* = 0.002368	14.275 ± 1.424 14.1 (6.51; 19.4) *p* = 0.000567
MDA in 1 % erythrocyte hemolysate (nM/ml)	4.539 ± 0.363 4.54 (3.1; 5.651)	7.249 ± 0.734 5.5 (4.191; 7.25) *p* = 0.010766	7.564 ± 0.801 6.186 (4.409; 7.767) *p* = 0.008801
Diene conjugates in blood plasma (U/ml)	0.296 ± 0.041 0.21 (0.19; 0.37)	1.313 ± 0.156 0.9 (0.43; 2.29) *p* = 0.000011	1.055 ± 0.182 0.71 (0.355; 1.055) *p* = 0.003255
Diene conjugates in 20 % erythrocyte hemolysate (U/ml)	0.18 ± 0.028 0.12 (0.09; 0.22)	1.192 ± 0.168 0.76(0.14; 2.15) *p* = 0.000038 1) 0.171 ± 0.03 (20) 0.14 (0.1; 0.19) 2) 1.921 ± 0.192 (28) 1.9 (1.035; 2.7) *p* = 0.000000	0.229 ± 0.04 0.14 (0.1; 0.24) p^1 = 0.000000

Note: statistical significance of differences from parameters in healthy women – *p*, from parameters in CC patients before surgery – p^1.

Superoxide radicals directly activate the reactions of xenobiotic metabolism and prostaglandin synthesis, participate in the expression of certain genes and cell proliferation. A number of studies have shown that SOD, in addition to its antioxidant role, performs a regulatory function, while being the main link in ensuring a constant oxygen concentration. A change in superoxide dismutase activity can cause various pathological processes [24]. Thus, a decrease in the intensity of enzymatic reactions leads to insufficient protection from active forms of oxygen metabolites. And an increase in the activity of SOD contributes to the cytotoxic effect of hydrogen peroxide formed as a result of oxygen dismutation [14]. In our study, it was determined that the initial values of SOD in patients with CC compared with the value in the group of women without oncopathology had statistically significant differences. The activity of SOD erythrocytes in patients with CC was reduced by 30–31 % (p = 0.000000) both before treatment and after surgery in comparison with the "healthy" group. A plasma study of the total activity of SOD

Table 2. Parameters of the enzymatic unit of the antioxidant defense in the blood of healthy women and CC patients before treatment and after the surgery

Index *M ± m* Me (Q25; Q75)	Study group		
	Healthy women, *n* = 26	CC patients before treatment, *n* = 48	CC patients after surgery, *n* = 48
SOD in erythrocytes (act. U/ml)	110.79 ± 4.29 113.95 (101.1;125.9)	76.05 ± 3.12 72.16 (63; 89.8) *p* = 0.000000	77.54 ± 3.57 80.5 (56.6; 95.15) *p* = 0.000000
SOD in blood plasma (act. U/ml)	0.023 ± 0.002 0.022 (0.014; 0.032)	0.026 ± 0.0018 0.026 (0.015;0.033)	0.017 ± 0.0007 0.016 (0.012; 0.02) *p* = 0.000459 p^1 =0.000005
Catalase in erythrocytes (μM H_2O_2/min×mg Hb)	2673.8 ± 60.3 2720.5 (2500;2846.9)	2093.8 ± 131.2 2100 (1965.6;2491) *p* = 0.000069	2464.6 ± 129 25373 (2034.8;2689) p^1 = 0.052306
Catalase in blood plasma (μM H_2O_2/min)	52.02 ± 2.91 50.95 (42.01; 59.5)	58.11 ± 3.73 57.49 (46.25; 71.4)	69.62 ± 6.38 66.47 (52.22; 80.5) *p* = 0.010340
Reduced glutathione (μM/mg Hb)	35.81 ± 1.81 34.97 (27.96; 43.82)	32.67 ± 2.88 32.31 (23.61;38.05)	31.01 ± 2.42 29.86 (25.51; 40.05)
Glutathione reductase (IU/mg hemoglobin)	7.348 ± 0.94 7.095 (3.47; 10.97)	6.066 ± 0.758 5.28 (3.49; 7.69)	7.239 ± 1.053 7.385 (2.385; 10.23)
Glutathione peroxidase (IU/mg hemoglobin)	232.9 ± 23.7 218.6 (118.7; 344.4)	471.9 ± 42.9 447.7 (317.1; 624.7) *p* = 0.000005	380.5 ± 31.7 405.9 (307.8; 469.1) *p* = 0.000440 p^1 = 0.095117
Glutathione transferase (IU/mg hemoglobin)	69.97 ± 5.29 64.02 (49.03; 77.18)	61.35 ± 3.88 59.2 (52.74; 68.45)	61.15 ± 4.47 58.95 (54.18; 70.42)

Note: statistical significance of differences from indices in healthy women – p, from indices in CC patients before surgery – p^1.

revealed a decrease in patients with CC after surgery by 26.1–34.6 % ($p < 0.001$) compared with healthy women and the indicator for CC before treatment (Table 2).

Catalase is the main components of the first line of antioxidant protection, which, being an enzyme coupled with superoxide dismutase, decomposes hydrogen peroxide formed during the dismutation of the superoxide radical. The maximum accumulation of the enzyme was registered in erythrocytes. In the interstitial fluid of the body, catalase has no long-term activity, which is explained by the result of the action of proteolytic enzymes. It is believed that outside of erythrocytes, the enzyme does not have an obvious protective function. At the same time, a number of pathological conditions with the manifestation of the inflammatory process are characterized by an increased content of catalase, which provokes a decrease in the intensity of oxidation processes of functionally important structures [12]. In our study, catalase activity was analyzed in healthy patients and in patients with CC. Thus, in patients with CC, we revealed an initial decrease in catalase activity in erythrocytes by 21.9 % compared with the group of healthy women ($p < 0.001$) and an increase in indicators, and in plasma in the early postoperative

Table 3. Levels of vitamins E and A in the blood plasma and erythrocytes in healthy women and CC patients before treatment and after the surgery

Index *M ± m* Me (Q25; Q75)	Study group		
	Healthy women, *n* = 26	CC patients before treatment, *n* = 48	CC patients after surgery, *n* = 48
Vitamin E (erythrocytes) (U/ml)	0.177 ± 0.010 0.15 (0.14; 0.19)	0.266 ± 0.022 0.22 (0.17; 0.37) *p* = 0.002497	0.462 ± 0.037 0.470 (0.20; 0.73) *p* = 0.000000 p^1 = 0.000010
Vitamin E (plasma) (U/ml)	0.474 ± 0.011 0.465 (0.44; 0.54)	0.274 ± 0.019 0.27 (0.16; 0.36) *p* = 0.000000	0.346 ± 0.034 0.34 (0.22; 0.47) *p* = 0.003036 p^1 = 0.057561
Vitamin A (erythrocytes) (U/ml)	0.212 ± 0.010 0.20 (0.19; 0.26)	0.219 ± 0.022 0.13 (0.12; 0.40)	0.109 ± 0.010 0.115 (0.07; 0.15) *p* = 0.000000 p^1 = 0.000026
Vitamin A (plasma) (U/ml)	0.759 ± 0.022 0.78 (0.62; 0.87)	0.252 ± 0.023 0.210 (0.125; 0.37) *p* = 0.000000	0.104 ± 0.020 0.075 (0.05; 0.1) *p* = 0.000000 p^1 = 0.000007
E/A coefficient (erythrocytes)	0.978 ± 0.122 0.70 (0.538; 0.95)	1.461 ± 0.131 1.50 (0.405; 1.923) *p* = 0.012006	4.903 ± 0.519 3.286 (2.9; 6.635) *p* = 0.000000 p^1 = 0.000000
E/A coefficient (plasma)	0.635 ± 0.017 0.64 (0.59; 0.71)	6.013 ± 0.807 4.00 (3.00; 8.00) *p* = 0.000000	4.915 ± 0.390 5.00 (3.30; 5.80) *p* = 0.000000
Erythrocytes/plasma vitamin A ratio	0.383 ± 0.0285 0.333 (0.295; 0.422)	0.946 ± 0.074 0.881 (0.515;1.375) *p* = 0.000000	1.382 ± 0.097 1.353 (1.00; 1.851) *p* = 0.000000 p^1 = 0.098750
Erythrocytes/plasma vitamin E ratio	0.284 ± 0.014 0.321 (0.227; 0.333)	1.139 ± 0.107 1.00 (0.333; 2.00) *p* = 0.000000	1.496 ± 0.097 1.7958 (1.00; 2.00) *p* = 0.000000 p^1 = 0.016233

Note: statistical significance of differences from indices in healthy women – *p*, from indices in CC patients before surgery – p^1.

period by 24.4 % (p = 0.01) (Table 2). This suggests the release of an insignificant amount of the enzyme from erythrocytes into the blood plasma due to the possible destabilization of the membranes of blood cells as a result of surgical exposure.

The study of the indicators of glutathione and glutathione-dependent enzymes in the blood of patients with CC allows us to determine the activity of this line of the antioxidant system, with a possible prognosis of the course of cancer. Being an active link in the mechanism of the cellular antioxidant defense system, glutathione is considered as one of the main components in the pathophysiology of cancer [12]. The reduced form of glutathione in the form of tripeptide – Lyglutamyl-Lcysteinylglycine is present in cells of various types. In this compound, the presence of a sulfhydryl group and a gamma-glutamyl bond, being an electron donor, collectively determines the functions of glutathione as a reducing agent of nucleic acids, protein molecules and lipids. This property of the chemical compound makes it possible to significantly reduce the level of ROS in non-enzymatic and enzymatic reactions. Intracellular imbalance of reduced and oxidized glutathione is observed in a number of pathologies, including the development of a malignant process [25]. The data obtained by us on the content of glutathione in the studied groups did not reveal statistical differences, although its level was slightly lower in patients with CC before and after surgery. A comparative analysis of glutathione-dependent enzymes revealed certain changes. Thus, the activity of glutathione peroxidase (GPO) in patients with CC significantly exceeded the level in the group of women without oncopathology by 102.9 % (p = 0.0002). After surgery, the trend did not change, the GPO indicators in patients with CC remained elevated by 63.4 % (p = 0.003) relative to healthy women. The study of the dynamics of glutathione reductase and glutathione transferase indices, significant changes in the group of patients with CC compared with the group of healthy ones did not reveal (Table 2).

Thus, in the group of patients with CC, before the start of treatment, there was a more than twofold increase in the activity of GPO against the background of a statistically significant decrease in catalase activity in erythrocytes, which indicated a switch in the decomposition of hydrogen peroxide and organic hydroperoxides to non-toxic metabolites from the catalase pathway to the glutathione peroxidase pathway. After surgery, there was a partial restoration of the ratio between catalase and GPO, characteristic of women without oncopathology: the catalase/ GPO ratio was normal 11.5; in patients with CC before surgery 4.4; in patients with CC after surgery 6.5.

It should be borne in mind that glutathione-dependent enzymes perform an antioxidant function and are triggers in the regulation of peroxidation mechanisms. It is fair to note that the role of some enzymes in the formation of the malignant process is not unambiguous. Organic hydroperoxides are mediators of most physiological processes, actively participating in the regulation of cell proliferation and apoptosis. There are publications on the role of the enzyme glutathione peroxidase with the detection of its activation in squamous cell carcinoma cells and in lung adenocarcinoma [16].

It is known that the lack of vitamin E in the body leads to the destabilization of cell membranes, which leads to the breakdown of unsaturated fatty acids, as well as to a violation of their protein composition. Another equally effective antioxidant is vitamin A, which on the one hand interacts with free radicals, and on the other hand provides a constant level of vitamin E, thereby contributing to its antioxidant effect [14]. Prior to the start of antitumor treatment in the blood plasma of patients with CC, the initial values of vitamin E and A in comparison with the group of conditionally healthy were reduced by 41.9 % and by 74.4 % (p = 0.000000). The content of vitamin E in erythrocytes was increased by 50.3 % (p = 0.0025), and no significant changes were found for vitamin A. In the postoperative period, an increase in vitamin E indices was recorded in the erythrocytes of patients with CC by 2.7 times relative to women without pathology and by 1.8 times relative to the baseline level (p = 0.000000), while a decrease in the content of vitamin E in plasma was observed – by 27 % (p = 0.0030) relative to healthy. The level of vitamin A was reduced both in erythrocytes by 1.9 times and in plasma by 2.4 times (p = 0.000000) relative to healthy and by 2 and 2.4 times relative to the indicators before treatment (p < 0.0001). According to the results obtained, which generally do not contradict the literature data [26], the content of vitamin E in patients with CC is initially reduced only in blood plasma. At the same time, the values of the ratio of vitamins E and A (E/A) in

patients with CC of reproductive age before the start of antitumor treatment were statistically significantly increased relative to those in healthy women, with an increase in red blood cells by 1.6 times (p = 0.015) and 9.5 times (p = 0.011) in blood plasma. Data on changes in the E/A coefficient in oncogynecological patients are consistent with the results of previously published data. Thus, in the publication of E. M. Franzyants (1995), an increase of 2–5 times in the E/A coefficient in blood cells in patients with different tumor localization in the dynamics of radiation therapy was shown. When analyzing the results of the erythrocyte/plasma ratio (the ratio of the level of vitamins in erythrocytes to their plasma content), we revealed a significant increase in this coefficient for both vitamins relative to the values in healthy women, before surgery, the increase in the erythrocyte/plasma ratio for vitamin A was 2.5 times, for vitamin E 4 times (p = 0.000000). After the operation, a similar pattern was observed – the ratio of vitamins E and A in patients with CC exceeded 5.3 times in red blood cells, and in blood plasma by 7.7 times relative to the indicators in healthy women. The erythrocyte/plasma ratio in patients diagnosed with CC in the postoperative period had a statistically significant increase. The increase for vitamin E was 5.1 times, for vitamin A – 3.8 times (p < 0.0001). These changes in the erythrocyte/plasma coefficients for vitamins E and A were regarded by us as a manifestation of hyperpolarization of erythrocyte membranes in cancer patients diagnosed with CC. According to the literature, a recorded increase in the erythrocyte/plasma coefficients for vitamins A and E was described in patients with CC during chemotherapeutic exposure with no obvious clinical effect, which indicated the destabilization of erythrocyte membranes in this category of oncogynecological patients compared with a pronounced antitumor effect in patients with CC [26].

CONCLUSION

As a result of the study, it was found that in patients with CC at the initial stages of the disease, significant changes were detected in the intensity of the processes of LPO and in the antioxidant system. We found a significant increase in the level of MDA and diene conjugates, a decrease in the activity of SOD and catalase, at the same time, increased initial activity of GPO, as well as low levels of vitamin E and A. Undoubtedly, these results complement the understanding of the processes occurring in the body of an oncological patient at the initial stage of tumor formation, which does not yet have an obvious clinical manifestation. After total removal of the ovaries, most of the indicators characterizing the enzymatic link of the antioxidant system tend to normalize, while the violation of the content of vitamins E and A (related to the non-enzymatic link of the antioxidant system) worsens. Desynchronization of the processes of free radical oxidation with multidirectional changes in the processes of oxidation and antioxidation in patients with early-stage CC at the stage of radical surgical treatment should be considered from the perspective of hormone-reducing surgery and the associated complex of changes in the organs and systems of women.

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ORIGINAL ARTICLE

SOLVOTHERMAL SYNTHESIS OF RHOMBIC SHAPE $GDF_3:TB^{3+}$ NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

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ABSTRACT

Purpose of the study. In this work, we have investigated the mechanism of structure formation of $GdF_3:Tb^{3+}$(15 %) nanocrystals synthesized by solvothermal synthesis in the temperature range from RT to 200 °C with a step of 50 °C.
Materials and methods. Nanocrystals of $GdF_3:Tb^{3+}$(15 %) were synthesized by the solvothermal method using a high-pressure reactor (autoclave) designed for temperatures up to 250 °C. The structure, size and morphology were determined by transmission electron microscopy (TEM), the type of crystal lattice and the size of crystallites of nanoparticles were studied by X-ray diffraction (XRD), hydrodynamic size of nanoparticles, particle size distribution, ζ-potential, agglomeration of nanoparticles in colloidal solutions were determined by dynamic light scattering (DLS), the chemical composition of the nanocrystals surface was studied by Fourier-transform infra-red spectroscopy (FT-IR), the nanoparticles ability to absorb UV radiation was analyzed by UV-visible spectroscopy (UV-vis) and X-ray excited optical luminescence (XEOL).
Results. With an increase in the temperature of the synthesis reaction, a structural change in the crystallites phase occurs from hexagonal to orthorhombic. At low temperatures, agglomerated particles consisting of hexagonal nanocrystals are formed, while at a temperature higher than the boiling point of the solvent, monodisperse rhombic-shaped nanoparticles with orthorhombic phase are formed. At mild temperatures, agglomerated particles with different morphology and with mixed hexagonal and orthorhombic phases are formed. Based on the analysis of X-ray spectrum, it was found that the size of $GdF_3:Tb^{3+}$(15 %) nanocrystals varies from 10 to 50 nm for different synthesis temperature conditions (T = RT, 50 °C, 100 °C, 150 °C, 200 °C). The hydrodynamic size of nanoparticles decreases with increasing synthesis temperature. All $GdF_3:Tb^{3+}$(15 %) nanocrystals obtained at different temperatures are transparent to visible light and absorb UV radiation. Absorption in the UV region increases with an increase in the size of particle crystallites. Upon X-ray irradiation of the colloidal $GdF_3:Tb^{3+}$(15 %) solution, X-ray excited optical luminescence spectra showed emission peaks at 490 nm, 543 nm, 585 nm and 620 nm.
Conclusion. The mechanism of structure formation of rhombic-shaped $GdF_3:Tb^{3+}$(15 %) nanocrystals has been investigated. These monodisperse rhombic-shaped nanoparticles can be used for X-ray induced photodynamic therapy (X-PDT) of superficial, solid and deep-seated tumors.

Keywords: solvothermal synthesis, GdF_3, Tb doped, scintillating nanoparticles, biomedical application, PDT, X-PDT

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ОРИГИНАЛЬНАЯ СТАТЬЯ

СОЛЬВОТЕРМАЛЬНЫЙ СИНТЕЗ НАНОЧАСТИЦ GDF_3:TB^{3+} РОМБИЧЕСКОЙ ФОРМЫ ДЛЯ БИОМЕДИЦИНСКИХ ПРИМЕНЕНИЙ

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РЕЗЮМЕ

Цель исследования. Исследовать механизм формирования нанокристаллов GdF_3:Tb^{3+}(15 %), полученных методом сольвотермического синтеза в интервале температур от комнатной температуры до 200 °C с шагом 50 °C.
Материалы и методы. Нанокристаллы GdF_3:Tb^{3+}(15 %) были синтезированы сольвотермальным методом с помощью реактора высокого давления (автоклав) рассчитанного на температуру до 250 °C. Структуру, размер и морфологию наночастиц исследовали методом просвечивающей электронной микроскопии (ПЭМ), тип кристаллической решетки и размер кристаллитов наночастиц определяли методом рентгеновской дифракции (РФА), гидродинамический размер наночастиц, гранулометрический состав, ζ-потенциал, агломерацию наночастиц в коллоидных растворах определяли методом динамического рассеяния света (ДРС), химический состав поверхности нанокристаллов изучали методом инфракрасной спектроскопии (ИК-спектроскопия), способность наночастиц поглощать УФ-излучение анализировали методом спектроскопии в видимой и УФ-областях спектра и рентгеновской оптической люминесценции.
Результаты. С повышением температуры реакции синтеза происходит структурное изменение фазы кристаллитов с гексагональной на орторомбическую. При низких температурах сольвотермального синтеза образуются агломерированные частицы, состоящие из гексагональных нанокристаллов, при температуре выше температуры кипения растворителя – монодисперсные наночастицы ромбической формы с орторомбической фазой. При умеренных температурах образуются агломерированные частицы различной морфологии со смешанной гексагональной и орторомбической фазами. На основании анализа рентгеновских спектров установлено, что размер нанокристаллов GdF_3:Tb^{3+}(15 %) меняется для разных температурных условий синтеза (T = КТ, 50 °C, 100 °C, 150 °C, 200 °C) от 10 до 50 нм. Гидродинамический размер наночастиц уменьшается при увеличении температуры синтезы. Все нанокристаллы GdF_3:Tb^{3+}(15 %) полученные при разных температурах прозрачны для видимого света и поглощают УФ-излучение. Поглощение в УФ области увеличивается при увеличении размера кристаллитов частиц. Спектры оптической люминесценции с возбуждением рентгеновским излучением (XEOL) показали пики излучения в видимом диапазоне на длинах волн 490 нм, 543 нм, 585 нм и 620 нм.
Заключение. Исследован механизм формирования нанокристаллов GdF_3:Tb^{3+}(15 %) ромбической формы. Монодисперсные наночастицы GdF3: Tb3+(15 %) ромбовидной формы могут найти применение для рентгеноиндуцированной фотодинамической терапии (ФДТ) поверхностных, а также объемных и глубоколежащих опухолей.

Ключевые слова: сольвотермальный синтез, GdF_3, легированный Tb, сцинтилляционные наночастицы, биомедицинское применение, ФДТ, Рентгеновская ФДТ

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BACKGROUND

Currently, photodynamic therapy (PDT) [1] and X-ray-induced PDT are modern therapeutic methods for the treatment of superficial, as well as volumetric and deep-lying tumors [2; 3]. A key role in the therapeutic effect of PDT is played by a photosensitizer, which selectively accumulates in the tumor tissue and, when irradiated with visible or near-infrared radiation of a certain wavelength, generates the formation of reactive oxygen species (ROS), which, in turn, kill cancer cells. For the X-ray-induced PDT (R-PDT) method, it is necessary to use scintillation nanoparticles that will effectively convert X-ray radiation into visible or near-infrared light with a certain wavelength to excite a photosensitizer [4; 5].

Gadolinium (III) fluoride is a multifunctional material with effective luminescence, excellent magnetic properties and low phonon energy, high chemical and thermal stability. Gadolinium fluoride nanoparticles doped with rare earth elements (Tb, Eu, etc.) can be used as effective converters for the R-PDT method and effectively convert X-rays into visible light with a certain wavelength. The large atomic number of gadolinium makes it possible to effectively absorb X-rays, so gadolinium fluoride nanoparticles can be used as a contrast agent for CT imaging. In addition, GdF_3 nanoparticles can be used in MRI due to their paramagnetic properties. When irradiated with both UV radiation and X-rays, GdF_3 nanoparticles doped with Tb^{3+} have strong green emission with a maximum at 545 nm and less intense satellite peaks at ~490, 585 and 620 nm due to electronic transitions from the excited state of $^5D_4$ to ^7F_J (J = 6–3) of the ground states of the Tb^{3+} ion [6].

Gadolinium trifluoride nanoparticles were obtained by several synthesis methods, including co-deposition [7], hydrothermal synthesis [6], solvothermal synthesis [8], microwave synthesis [10]. For example, Zhang et al. synthesized GdF_3:Eu^{3+} nanoluminophores with a hexagonal or orthorhombic structure at room temperature using the chemical co-deposition method [7]. The structure and morphology of GdF_3:Eu^{3+} nanoluminophores were controlled using various fluorine precursors. Hexagonal GdF_3:Eu^{3+} nanocrystals were formed using $NaBF_4$ as a fluoride precursor, whereas orthorhombic GdF_3:Eu^{3+} nanocrystals were obtained using a NaF or NH4F fluoride precursor. It has also been experimentally established that hexagonal GdF_3:Eu^{3+} nanoluminophores emit significantly stronger Eu3+ luminescence than orthorhombic ones. Samantha et al. A simple microwave method was reported for the synthesis of stable Eu^{3+}-doped GdF_3 nanocrystals with a hexagonal phase functionalized with polyvinylpyrrolidone at higher temperatures (up to 220 °C), achieved by adjusting the viscosity of solvents, as well as using KF as a source of fluorine [8]. Both the morphology and the size of GdF_3 nanocrystals can also be varied by adjusting the reaction conditions. Wang et al. various monodisperse colloidal nanocrystals GdF3: Yb, Er with increased frequency with different shapes, sizes and alloying impurities were synthesized using microwave synthesis [10]. In addition to highly monodisperse spherical particles, they prepared monodisperse slices of rhombic shape, showing a tendency to self-assemble into stacks. Sui et al. [9] reported the behavior of the orthorhombic REF_3 phase at high pressure (RE = Sm to Lu and Y). Pressure-induced GdF_3 phase transitions were studied at room temperature. It is established that the pressure range of the phase transition from the orthorhombic to the hexagonal phase is 5.5–9.3 GPa for GdF3.

In this paper, the mechanism of formation of GdF_3:Tb^{3+}(15 %) nanocrystals synthesized by solvothermic synthesis in the temperature range from room temperature to 200 °C. The physicochemical properties were studied by transmission electron microscopy (TEM), X-ray diffraction (XRD), dynamic light scattering (DRS), infrared spectroscopy, spectroscopy in the visible and UV spectral regions and X-ray optical luminescence.

MATERIALS AND METHODS

Gadolinium nitrate hexahydrate $Gd(NO_3)_3 \cdot 6H_2O$ (99.9 %) and terbium chloride hexahydrate $TbCl_3 \cdot 6H_2O$ (99.9 %), ammonium fluoride NH_4F (98 %) were purchased from Alfa Aesar (Haverhill, Massachusetts, USA). Ethylene glycol was purchased from Sigma-Aldrich (Burlington, Massachusetts, USA). All chemicals were used without additional purification.

The physicochemical characteristics of GdF_3:Tb^{3+}(15 %) nanocrystals (T = RT, 50 °C, 100 °C, 150 °C, 200 °C) were determined by the following experimental methods. The size, shape and morphology were studied using TEM on a Tecnai G2 Spirit BioTWIN device (FEI, USA). The type of crystal lattice

and the average size of nanoparticle crystallites were determined by XRD on a D2 PHASER diffractometer (Bruker Corp., Germany). The hydrodynamic size of nanoparticles, granulometric composition, ζ-potential, and agglomeration of nanoparticles in colloidal solutions were determined by DRS on a NANO-Flex particle size analyzer (MicroTrac GmbH, Germany) and STABINO (ParticleMetrix, USA). The quantitative and qualitative chemical composition and concentration of alloying elements were evaluated using a two-dimensional microrentgenofluorescence (XFA) spectrometer M4 Tornado (Bruker Corp., Germany). The surface chemistry was studied using infrared Fourier spectroscopy (FTIR) on a Vertex 70 spectrometer (Bruker Corp., Germany). Emission spectra (XEOL) of nanomaterial powders and colloidal aqueous solutions were studied using a RAP-90U X-ray tube with a protective casing and a Shimadzu UV-2600 dual-beam spectrophotometer (Shimadzu, Japan).

GdF_3:Tb^{3+} nanocrystals synthesis

GdF_3:Tb^{3+} nanocrystals (15 %) were obtained by the solvothermal synthesis method. To obtain 160–200 mg of GdF_3:Tb^{3+} nanocrystal powder (15 %), it is necessary to: dissolve 0.85 mmol Gd $(NO_3)_3H_2O$ (*m* = 0.384 g) and 0.15 mmol TbCl3·C2·6H2O (*m* = 0.056 g) in 10 ml of ethylene glycol (EG) in a beaker at room temperature. For better dissolution of chemical reagents, ultrasonic dispersants can be used. After mechanical stirring for about 1 hour, add 3 mmol NH4F (*m* = 0.1111 g), previously dissolved in 10 ml of ethylene glycol (EG), drop by drop. During the reaction, the previously transparent solution becomes cloudy and white due to the deposition of doped gadolinium fluoride. Further, the resulting solution was subjected to heat treatment in a Teflon autoclave in the temperature range from RT to 200 °C with intensive stirring for 24 hours. The final product was washed three times with distilled water using centrifugation. After the last centrifugation, the white nanocrystals were dried in a drying cabinet at 60 °C. The obtained nanocrystals were denoted respectively GdF_3 GdF_3:Tb^{3+} (15 %) (T = RT, 50 °C, 100 °C, 150 °C, 200 °C). Colloidal aqueous solutions of GdF_3:Tb^{3+} nanocrystals were prepared by dispersing nanocrystals in bidistilled water using an ultrasonic disperser.

RESEARCH RESULTS AND DISCUSSION

We have studied the mechanism of formation of GdF_3:Tb^{3+} (15 %) nanocrystals (T = RT, 50 °C, 100 °C, 150 °C, 200 °C) obtained by the solvothermal synthesis method in the temperature range fromRT to 200 °C in increments of 50 °C. The solvothermal method is a chemical reaction occurring in a solvent at a temperature above the boiling point of the solvent (usually < 250 °C) in a sealed reactor. Ethylene glycol with a boiling point of 197 °C was used as a solvent. By varying the synthesis parameters: temperature and reaction time, this method makes it possible to obtain nanocrystals with size control, morphology and a high level of crystallinity.

Figure 1a shows diffractograms in the range of 22°–32° degrees of nanocrystals obtained during synthesis at various reaction temperatures (T = RT, 50 °C, 100 °C, 150 °C, 200 °C) for 24 hours. It is established that with an increase in the temperature of the synthesis reaction, the structure of nanocrystals undergoes a structural change from the hexagonal to the orthorhombic phase. At a synthesis temperature of 50 °C, a purely hexagonal structure is observed, and at a temperature of 200 °C a pure orthorhombic structure with no secondary phases is already observed. At moderate temperatures, a mixed phase of hexagonal and orthorhombic phases is observed. Figure 1b shows diffractograms of nanocrystals of hexagonal and orthorhombic phases. The position of the peaks and their intensity correspond exactly to the diffractograms of orthorhombic GdF_3 (ICSD chart 00-012-0788) and hexagonal SmF3 (ICDS chart PDF No. 01-072-01439). No additional peaks of any secondary phases were detected.

Based on the Scherrer equation, reflex broadening was used to estimate the average size of crystallites. The average size of crystallites in GdF_3:Tb^{3+} nanocrystals (15 %) varies from 10 nm to 50 nm for different synthesis reaction temperatures: from room temperature (RT), 50 °C, 100 °C, 150 °C, 200 °C. X-ray fluorescence analysis (XFA) confirmed the chemical composition of Tb/Gd (15 %) for all synthesized nanocrystals, which indicates good solubility of rare earth element salts in the process of solvothermal synthesis. According to the data of dynamic light scattering in colloidal solutions of nanocrystals, the hydrodynamic radius of nanoparticles gradually decreases from 220 ± 200 nm for RT, 174 ± 90 nm

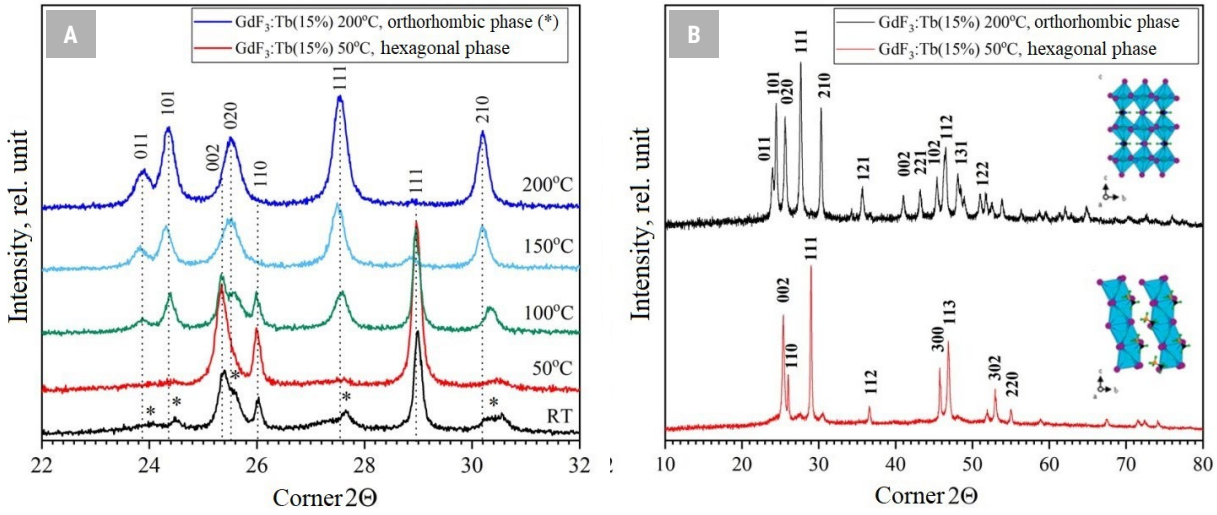

Fig. 1. a) GdF_3:Tb^{3+} diffractograms of nanocrystals (15 %) (T = RT, 50 °C, 100 °C, 150 °C, 200 °C); b) GdF_3:Tb^{3+} diffractograms (15 %) (T = 50 °C) of hexagonal phase and GdF_3:Tb^{3+} (15 %) (T = 200 °C) orthorhombic phase.

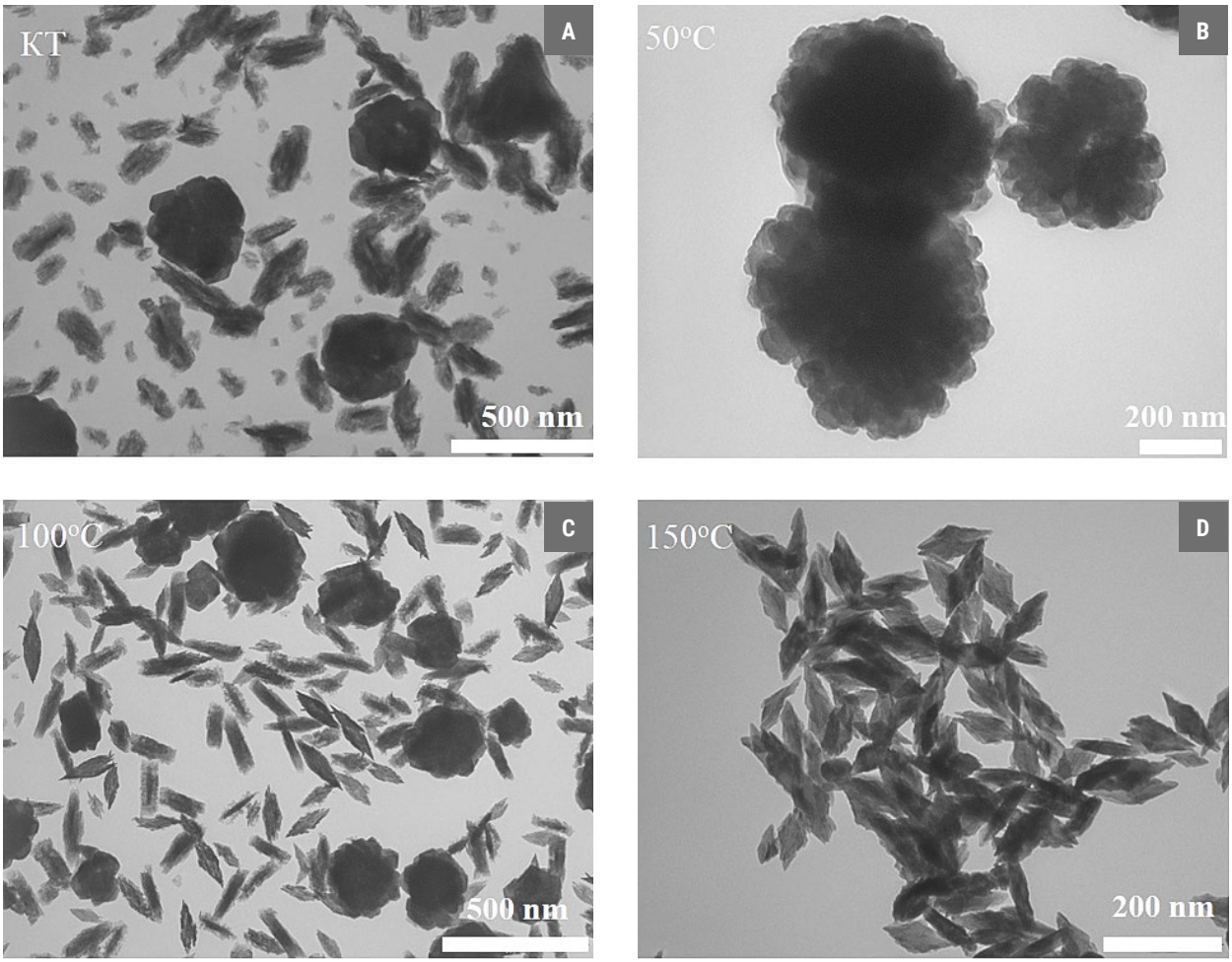

Fig. 2. a) GdF_3:Tb^{3+} nanocrystals TEM-imaging (15 %) (RT); b) hexagonal phase GdF_3:Tb^{3+} nanocrystals TEM-imaging (15 %) (50°C); c) GdF_3:Tb^{3+} nanocrystals TEM-imaging (15 %) (100°C); d) GdF_3:Tb^{3+} rhombic shape with orthorhombic phase nanocrystals TEM-imaging (15 %) (150°C).

for 50 °C, 150 ± 116 nm for 100 °C, 57 ± 39 nm for 150 °C, to 48 ± 32 nm for 200 °C. The decrease in the hydrodynamic radius is associated with the recrystallization of nanocrystallites during heat treatment during synthesis and the reduction of the interparticle space in agglomerated particles.

Figure 2 shows TEM images of nanocrystals obtained at various synthesis temperatures (RT 50 °C, 100 °C, 150 °C). Figure 2a shows agglomerated particles of various morphologies synthesized at room temperature (RT) with an average size of 150–180 nm, consisting of small orthorhombic phase nanoparticles up to 10 nm in size. There are also large agglomerated particles consisting of hexagonal particles up to 50 nm in size, with well-defined faces and good crystallinity. Figure 2b shows large agglomerated particles up to 500 nm in size in the form of "flowers" consisting of hexagonal phase crystallites. The crystallites have a hexagonal shape and a size of 30–50 nm. Figure 2b shows agglomerated nanoparticles obtained at a reaction temperature of 100 °C in the form of "flowers" and rhombic and spindle-shaped particles. In these types of agglomerated nanoparticles, regions with higher and lower densities are noticeable. A higher temperature is required for the formation of rhombic nanoparticles with good crystallinity. The image also contains agglomerated nanoparticles up to 200 nm in size, rectangular and spindle-shaped, consisting of small crystallites up to 10 nm in size. Figure 2g shows nanoparticles

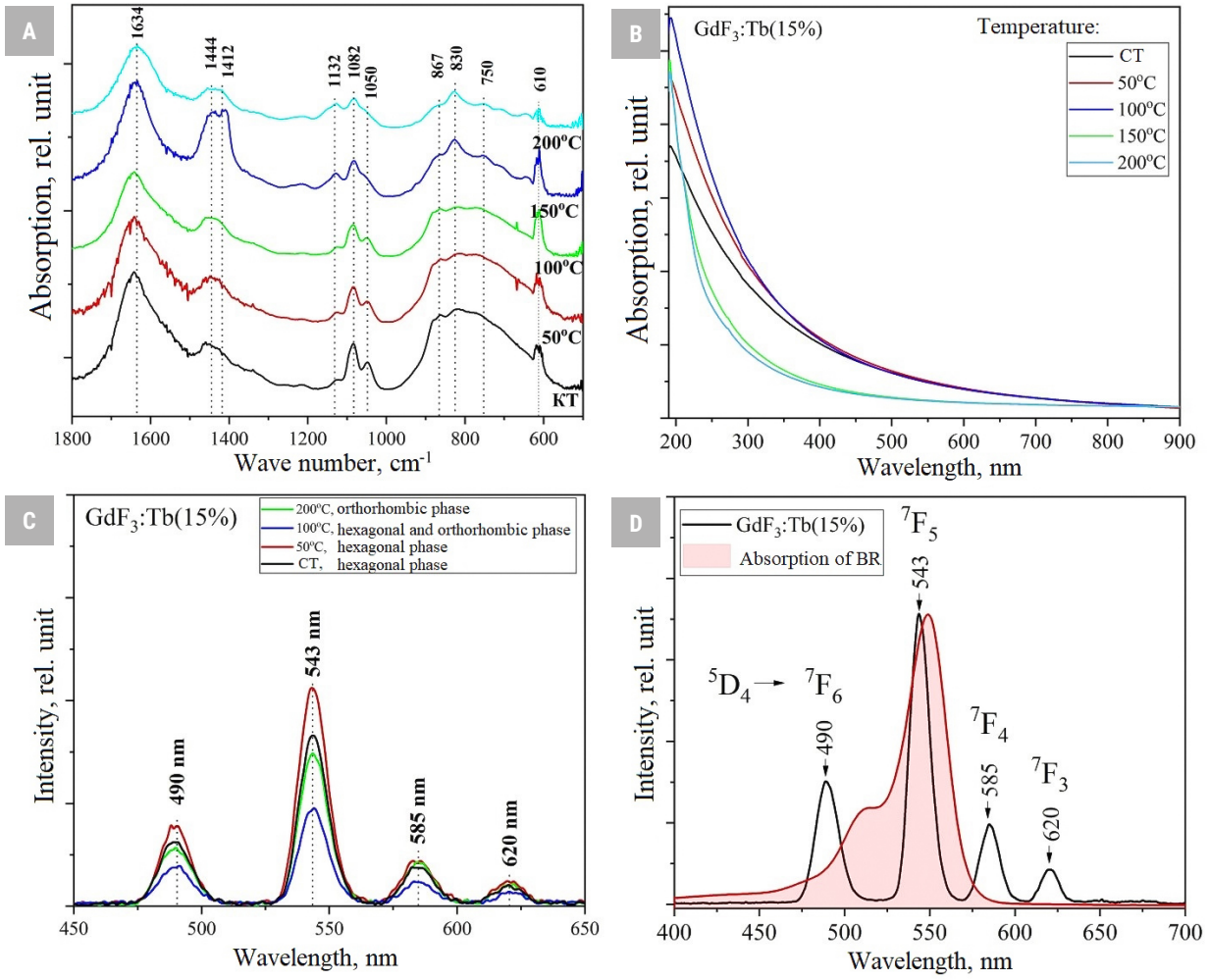

Fig. 3. Absorption spectrum in a) IR range; b) in visible and UV regions; c) optical luminescence spectrum with XEOL of GdF_3:Tb^{3+} (15 %) nanocrystals (T = RT, 50°C, 100°C, 150°C, 200°C); d) comparison of the spectrum of XEOL nanoparticles PEG@GdF_3:Tb^{3+}(15 %) excited by X-ray irradiation (35 kV, 16 mA), and the absorption spectrum of the UV-visible photosensitizer bengal pink (BP).

obtained at a reaction temperature of 200 °C in the form of rhombic nanoparticles.

Figure 3a shows the absorption spectra in the IR range of $GdF_3:Tb^{3+}$ nanocrystals (15 %) (T = RT 50 °C, 100 °C, 150 °C, 200 °C). Wide peaks in the region of 1600–1650 cm^{-1} and 650–950 cm^{-1} are associated with bending and vibrational modes of adsorbed water molecules on the surface of nanocrystals [5]. The valence and deformation vibrations C = O are at 1665 and 1436 cm^{-1}. The band at 610 cm^{-1} can be attributed to vibrations of the gadolinium fluoride lattice, which confirms the formation of gadolinium fluoride nanocrystals. Peaks ~1412 and 1444 cm^{-1} refer to methylene scissor and valence vibrations of C-O-C EG. UV-visible spectra of $GdF_3:Tb^{3+}$ nanocrystals (15 %) (T = RT, 50 °C, 100 °C, 150 °C, 200 °C) are shown in Figure 3b. All the obtained nanocrystals are transparent to visible light and absorb UV radiation. The absorption in the UV region increases with the increase in the size of the crystallites of the particles. Figure 3b shows optical luminescence spectra with XEOL of $GdF_3:Tb^{3+}$ nanocrystals (15 %) (T = RT, 50 °C, 100 °C, 150 °C, 200 °C). Fluorescence emission can be excited by both UV light and X-ray radiation, which gives the same typical Tb^{3+} emission profile. Strong green glow of scintillation nanocomposites PEG@$GdF_3:Tb^{3+}$ (15 %) with a main peak at 545 nm and three satellite peaks at 490, 585 and 620 nm is due to electronic transitions from the excited state of 5D4 to the ground states of Tb^{3+} ^7F_J ions (J = 6–3). Figure 3g shows a comparison of the spectrum of XEOL nanoparticles PEG@$GdF_3:Tb^{3+}$ (15 %) excited by X-ray radiation (35 kV, 16 mA), and the absorption spectrum of the bengal pink photosensitizer (BP).

CONCLUSION

In this paper, the mechanism of formation of $GdF_3:Tb^{3+}$ (15 %) nanocrystals obtained by solvothermal synthesis in the temperature range from RT to 200 °C. At low temperatures, agglomerated particles consisting of hexagonal nanocrystals are formed, and at temperatures above the boiling point of the solvent, monodisperse rhombic nanocrystals with an orthorhombic phase are formed. At moderate temperatures, agglomerated particles of various morphologies with mixed hexagonal and orthorhombic phases are formed. Under X-ray irradiation of a $GdF_3:Tb^{3+}$ (15 %) colloidal solution, optical luminescence spectra with XEOL showed radiation peaks at 490 nm, 543 nm, 585 nm and 620 nm. Monodisperse nanocrystals of rhombic shape can be used for X-ray induced photodynamic therapy (X-PDT) of surface, volume and deep-lying tumors.

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Pankin I. A. – conducting experiments;

Bulgakov A. N. – conducting experiments;

Rud P. A. – conducting experiments;

Soldatov A. V. – research concept, scientific guidance.

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ORIGINAL ARTICLE

CULTIVATION OF CELLS IN ALGINATE DROPS AS A HIGH-PERFORMANCE METHOD OF OBTAINING CELL SPHEROIDS FOR BIOPRINTING

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ABSTRACT

Purpose of the study. Testing the protocol of obtaining cell spheroids of breast cancer cell cultures for bioprinting by growing in alginate drops.

Materials and methods. Cells of breast cancer cell lines BT-20 and MDA-MB-453 were cultured in DMEM medium supplemented with 10 % FBS. Next, the cells were removed from the plastic using a trypsin-Versene solution and resuspended in a sterile 2 % alginate solution in DPBS to the concentration of 10^5 cells/ml. Then the alginate solution with the cells was slowly dripped through a 30G needle into a sterile cooled solution of calcium chloride (100 mM) from a height of 10 cm. After polymerization, alginate drops were washed in DMEM and cultured for two weeks in DMEM with the addition of 10 % FBS at 37 °C and 5.0 % CO_2.The spheroids formed in the alginate were photographed on the 3rd, 7th, 10th, and 14th days of cultivation, after which they were removed from the alginate by keeping in 55 mM sodium citrate solution with the addition of 20mM ethylenediaminetetraacetic acid (EDTA) and embedded in paraffin blocks according to the standard method, followed by histological examination.

Results. Cellular spheroids were formed in both cell cultures already on the 3rd day of cultivation. From the 3rd to the 10th day in both cultures, a uniform growth of cell spheroids was observed with a gradual slowdown in the increase in the size of spheroids by the 14th day of cultivation. On the 10th day the proportion of cells that formed clones (more than 500 μm^2 in size) was 25.2 % ± 7.1 % (n = 25) in the BT-20 culture and 38.5 % ± 9.9 % (n = 25) in MDA-MB-453 culture. On the 14th day, BT-20 culture was characterized by spheroids varying little in size and shape, with an average area of 1652 ± 175 μm^2, having a dense structure with smooth edges. The spheroids in MDA-MB-453 culture turned out to be more loose and easily deformed, their size and shape varied noticeably, the average area of the spheroids was 2785 ± 345 μm^2.

Conclusion. The production of spheroids in alginate drops is inferior in speed to the methods of forming cell conglomerates in hanging drops or on microwells, but it surpasses these methods in productivity, which is comparable to the production of spheroids by constant medium stirring on low-adhesive substrates. In addition, the clonal nature of the obtained spheroids leads to an increase in research costs and thus limits their scalability.

Keywords: 3D cell culture, cell spheroid, alginate, bioprinting

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3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

КУЛЬТИВИРОВАНИЕ КЛЕТОК В АЛЬГИНАТНЫХ КАПЛЯХ, КАК ВЫСОКОПРОИЗВОДИТЕЛЬНЫЙ МЕТОД ПОЛУЧЕНИЯ КЛЕТОЧНЫХ СФЕРОИДОВ ДЛЯ БИОПЕЧАТИ

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РЕЗЮМЕ

Цель исследования. Тестирование протокола получения клеточных сфероидов культур рака молочной железы (РМЖ) для биопечати путём наращивания в альгинатных каплях.

Материалы и методы. Клетки культур BT-20 и MDA-MB-453 культивировали в среде DMEM с добавлением 10 % FBS. Далее клетки снимали с пластика при помощи раствора трипсин-Версена и ресуспендировали в стерильном 2 % растворе альгината, приготовленном на DPBS, до концентрации 10^5 кл/мл. Раствор альгината с клетками исследуемых культур РМЖ медленно капали через иглу 30G в стерильный охлажденный раствор хлорида кальция (100 мМ) с высоты 10 см. После полимеризации альгинатные капли отмывали в среде DMEM и культивировали в течение двух недель в среде DMEM с добавлением 10 % FBS при 37 °C и 5,0 % CO_2. Образующиеся в альгинате сфероиды фотографировали на 3-, 7-, 10- и 14-е сутки культивирования, после чего их извлекали из альгината путём выдерживания в 55мМ растворе цитрата натрия с добавлением 20мМ этилендиаминтетрауксусной кислоты (ЭДТА) и заключали в парафиновые блоки по стандартной методике с последующим гистологическим исследованием.

Результаты. Клеточные сфероиды-клоны образовывались в обеих культурах уже на 3 сутки культивирования. С 3 по 10-е сутки в обеих культурах наблюдался равномерный рост клеточных сфероидов с постепенным замедлением увеличения размеров сфероидов к 14-му дню культивирования. Доля клеток, образовавших клоны (размером более 500 мкм^2), на 10-е сутки составила 25,2 % ± 7,1 % (n = 25) в культуре BT-20 и 38,5 % ± 9,9 % (n = 25) в культуре MDA-MB-453. На 14-е сутки для BT-20 были характерны сфероиды, мало варьирующие по размеру и форме, средней площадью 1652 ± 175 мкм^2, обладающие плотной структурой с ровными краями. Сфероиды из клеток культуры MDA-MB-453 оказались более рыхлыми и легко деформирующимися, их размеры и форма заметно варьировали, средняя площадь сфероидов составила 2785 ± 345 мкм^2.

Заключение. Получение сфероидов в альгинатных каплях уступает по скорости методам формирования клеточных конгломератов в висячих каплях или на микроячейках, однако превосходит эти методы по производительности, которая сравнима с получением сфероидов на низкоадгезивных подложках путём постоянного перемешивания среды. Кроме того, клональная природа получаемых сфероидов приводит к увеличению трат на проведение исследований и ограничивает, тем самым, их масштабируемость.

Ключевые слова: трёхмерная клеточная культура, клеточный сфероид, альгинат, биопечать

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BACKGROUND

Breast cancer (breast cancer) remains an urgent problem of modern healthcare [1; 2]. In the structure of the incidence of malignant neoplasms of the female population in Russia, breast cancer occupies the first place [3; 4], its incidence in 2021 accounted for 22.1 % of new cases, and the average annual rate of increase in morbidity from 2011 to 2021 was 1.72 %. In terms of mortality, this pathology also ranks first among malignant diseases in women – breast cancer accounted for 15.8 % of all cancer deaths in 2021 [5]. The reduction of mortality depends on the development and introduction of new drugs and approaches to the treatment of tumors. Despite the progress in the discovery of new antitumor agents, only a small part of the substances that have demonstrated effectiveness in preclinical studies on cell cultures and animal models are successfully undergoing clinical trials. Scientists agree that the main reason for this state of affairs is the discrepancy between the biological characteristics of monolayer cell cultures and tumors grown in animal models and the characteristics of human tumors [6]. In particular, it is known that the cultivation of cancer cells in two-dimensional cultures leads to a change in their phenotype and loss of expression of molecules of key signaling pathways, which these cells demonstrate *in vivo* in the body of patients [7]. In this regard, it is urgent to create new models of tumor growth that would combine the mass and reproducibility characteristic of *in vitro* cell cultures and the complexity demonstrated by animal models. One of the promising directions of the search is to recreate the three-dimensional microenvironment of the tumor *in vitro* by combining various components using bioprinting methods [8]. Researchers use various materials and approaches to the construction of such models, but in general they are malignant cells and microenvironment cells enclosed in biogels of different chemical nature and origin. In this case, most often the cells are located in the thickness of the biochernils singly, at a distance significantly exceeding that observed in situ [8]. Such a structure of the model obviously does not allow to fully reflect the biological features of the tumor, so attempts are being made to introduce cellular spheroids into bioprinted designs – the simplest models of tumor nodes that have proven themselves well in the practice of preclinical studies [9]. As a rule, cell spheroids for bioprinting are obtained by methods of mechanical agglomeration of cells by suspending a cell suspension in droplets, using small-cell plates or special matrices, culturing on a low-adhesive substrate with constant mixing of the medium, as well as by directly imprinting a biogel with a high concentration of cells into the matrix model [10]. One of the least common approaches to obtaining cellular spheroids for bioprinting is their cultivation from single progenitor cells. However, spheroids obtained in this way, in our opinion, are more adequate models of micrometastases or early stages of tumor node formation than mechanical cell conglomerates, since each cellular spheroid in this case is a clone of one cell. In addition, the tumor model assembled from individual clones is more consistent with the structure observed in a heterogeneous tumor in situ. As a rule, spheroids-clones are obtained by culturing tumor cells in a biogel that does not support cell adhesion. The most popular biogel for producing spheroids is soft agar or agarose gels, which are traditionally used by researchers to estimate the number of tumor stem cells (TSC) in culture [11]. However, to obtain spheroids suitable for bioprinting, this method is not applicable, since the extraction of cells from agarose gel is associated with heating and mechanical action, which reduces their viability. We suggested that encapsulation of cells in alginate droplets could be considered as a promising method for obtaining spheroids for bioprinting. Previously, this approach was tested by us to study the properties of stemness in adhesive cell lines of colorectal cancer [12]. Alginate gel, like agarose, does not support adhesion, but its advantage is the ability to rapidly depolymerize under the action of agents chelating calcium ions, which allows you to quickly extract the resulting spheroids under physiological conditions and then use them in bioprinting. Analysis of the literature data has shown that the approach based on the build-up of spheroids in alginate, followed by purification and conclusion of biogel, has not yet been applied by anyone. In some studies, bioprinting of a tumor model with single cells in an alginate-gelatin biogel is found, followed by the formation of spheroids-clones directly in the resulting structure [13]. In contrast to our proposed approach, this method of including spheroids in the model does not make it possible to widely vary the

composition of biochernils, and also does not allow for precise adjustment of the composition and location of other elements of the model.

The purpose of the study: development and testing of a protocol for obtaining spheroids of breast cancer cultures for bioprinting by building up in an alginate gel.

MATERIALS AND METHODS

BC cell cultures BT-20 and MDA-MB-453 served as the material for the study. The cells were cultured in DMEM (Gibco) medium with the addition of 10 % FBS (Hyclone). When the monolayer of cells reached 70 % confluence, they were removed from the plastic using a trypsin-Versene solution (1:1; Biolot, Russia). Next, the cells were resuspended in a sterile 2 % alginate solution (Sigma) prepared on DPBS (Biolot) to a concentration of 10^5 cells/ ml. The alginate solution with the cells of the studied breast cancer cultures was slowly dripped through a 30G needle into a sterile cooled calcium chloride solution (100 mM) from a height of 10 cm. Upon contact with a solution of calcium chloride, alginate droplets were instantly cured and beads with a diameter of 2.5–3 mm were formed (Fig. 1). The obtained beads were kept for additional polymerization in a solution of calcium chloride for another 5 minutes and washed once with a cooled DMEM medium, after which they were placed in a cultivation medium consisting of DMEM with the addition of 10 % FBS. Next, alginate beads with breast cancer culture cells enclosed in them were cultivated for 14 days with medium replacement every three days. Spheroids formed in alginate were photographed on the 3rd, 7th, 10th and 14th days of cultivation. In the obtained images, the size of the spheroids was determined using the ImageJ package. Statistical processing of the results was carried out using MS Excel software.

On the 14th day of cultivation, alginate was dissolved by holding beads in a 55mM sodium citrate solution with the addition of 20mM ethylenediaminetetraacetic acid (EDTA) for 3 minutes at room temperature. The isolated spheroids were washed twice in a culture medium and placed in a 5 % agarose gel, which, after curing, was enclosed in a paraffin block according to a standard procedure. The histological structure of the obtained cellular spheroids was studied on sections stained with hematoxylin-eosin.

RESEARCH RESULTS AND DISCUSSION

Both cell cultures in alginate drops showed growth already on the third day of cultivation. At the same time, the BT-20 culture formed approximately the same rounded spheroids in size, while the cells of the MDA-MB-453 culture formed spheroids of different shapes and sizes (Fig. 2a, b). The difference in the

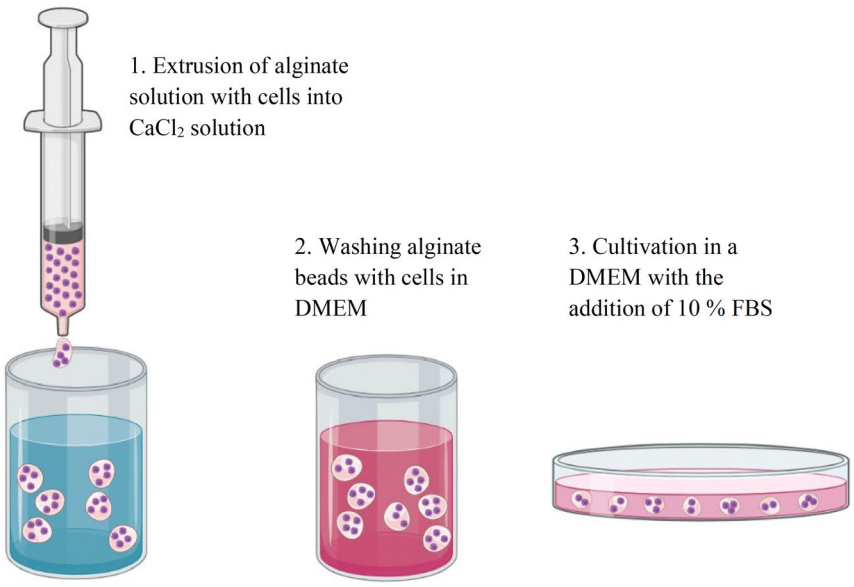

Fig. 1. Schematic representation of the protocol for obtaining cellular spheroids in alginate droplets.

growth pattern of spheroids remained on the 10th day of cultivation (Fig. 2b, d).

Variability in the size and shape of spheroids can be a negative phenomenon for those bioprinting applications in which measurement of the size of spheroids in the composition of the resulting contruct is required in response to various influences. As, for example, it is required in the case of bioprinting a tumor growth model. If compared with other methods of spheroid formation, the proposed method is inferior in this indicator to methods of direct imprinting of a thick suspension of cells into the matrix model and methods of controlled agglomeration of cells in hanging droplets or microlunks, but comparable to free-floating spheroids-conglomerates on low-adhesive substrates [4]. To overcome this disadvantage, it is possible to propose the use of various methods of separation (filtration, centrifugation) of spheroids-clones in size before printing or the use of automatic microscopes with precise positioning of the slide table for the analysis of the resulting models, which will allow taking into account the behavior of each spheroid separately.

From the 3rd to the 10th day of cultivation, uniform growth of cellular spheroids was observed in both cultures with a gradual slowdown in the increase in the size of spheroids by the 14th day of cultivation. At the same time, on average, the size of the MDA-MB-453 spheroids was larger than that of BT-20 (Fig. 3). For BT-20, spheroids with an average area of 1652 ± 175 μm^2 were characteristic, and the average area of spheroids formed by MDA-MB-453 culture cells was 2785 ± 345 μm^2 . This phenomenon can be explained by the semi-adhesive nature of the growth of the MDA-MB-453 culture on culture plastic, which gives the cells of this culture an advantage even under growing conditions in an alginate gel that does not support cell adhesion.

Thus, the optimal time for collecting spheroids for the purpose of further bioprinting for these crops is 7–10 days of cultivation. This rate of spheroid formation is comparable to free-floating spheroids-

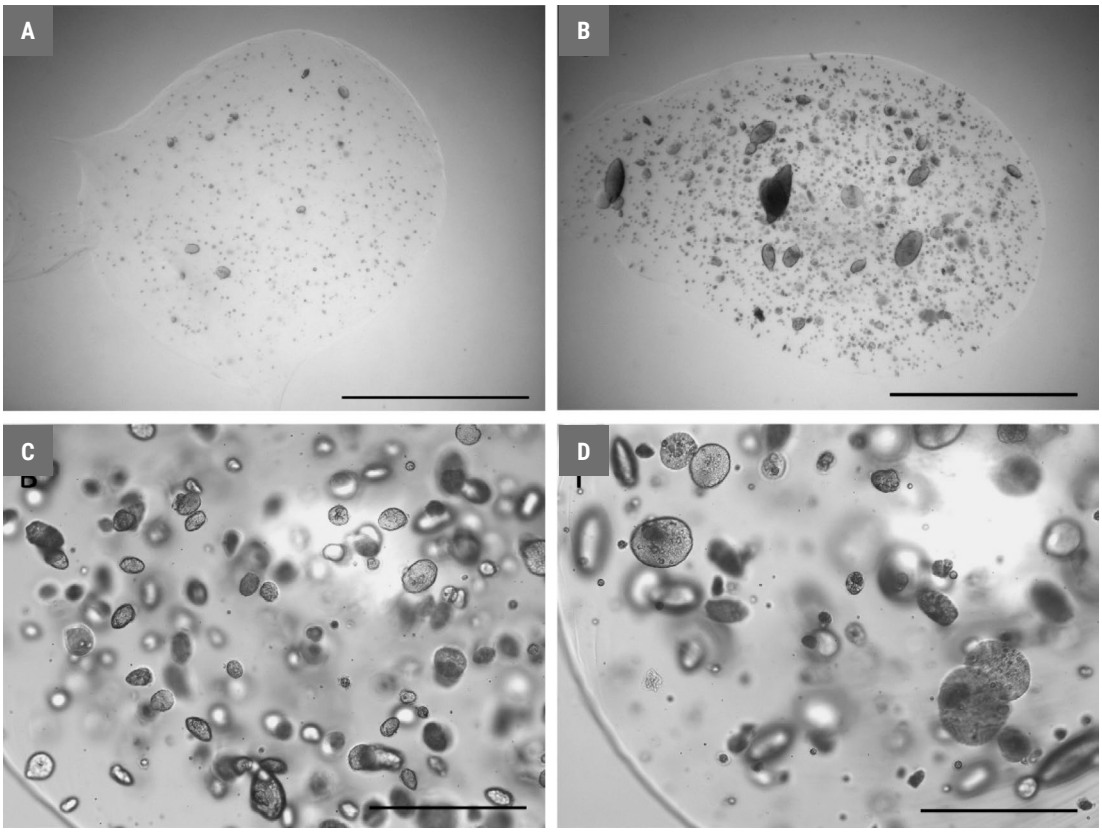

Fig. 2. Beads made of 2 % alginate gel with breast cancer culture cells enclosed in them. a – culture BT-20 for the 3rd cultivation knocks; b – culture MDA-MB-453 for the 3rd cultivation knocks; c – culture BT-20 for the 10th cultivation knocks; d- culture MDA-MB-453 for the 10th cultivation knocks. The size of the scale lines: a, b – 3 mm; c, d – 0.5 mm.

conglomerates on low-adhesive substrates, but significantly inferior to the rate of formation of cellular spheroids with controlled agglomeration of cells in hanging droplets or microlunks, where this indicator can range from 3 to 48 hours, depending on the materials used [10].

On the 10th day of cultivation, when the increase in the size of the spheroids begins to stabilize and it is possible to proceed to bioprinting, we measured the proportion of cells that formed clones (larger than 500 microns). Data analysis showed that this indicator was 25.2 % ± 7.1 % (n = 25) in BT-20 culture and 38.5 % ± 9.9 % (n = 25) in MDA-MB-453 culture. Thus, at a concentration of 100 thousand cells/ml, from one milliliter of alginate gel with BT-20 and MDA-MB-453 culture cells, 25–38 thousand spheroids suitable for further bioprinting can be obtained, which is orders of magnitude greater than the capabilities of even high-performance methods of spheroid formation in microlunks and hanging droplets and comparable to the formation performance free-floating spheroids-conglomerates on low-adhesive substrates [10]. The yield of cellular spheroids and their growth rate can be increased even more if growth factors such as EGF and FGF, which enhance cell division under conditions of reduced adhesion, are used [12].

The histological structure of spheroids after extraction from alginate gel differed between cultures. BT-20 was characterized by spheroids of dense structure with smooth edges, retaining their shape after extraction from the gel, double washing and inclusion in the agarose block (Fig. 4a). Spheroids from the cells of the MDA-MB-453 culture turned out to be more loose, easily deformed with the loss of part of the cells from the surface layers after washing in the culture medium (Fig. 4b). The observed differences may be associated with a smaller amount of extracellular matrix formed by cells of the MDA-MB-453 culture in comparison with the BT-20 culture.

The structure of spheroids plays an important role in bioprinting. Loose decaying cellular conglomerates lose their shape during extrusion through the nozzle, which negatively affects the quality of measurement results carried out on a 3d model of tumor growth obtained by bioprinting using such spheroids. Cellular conglomerates obtained by aggregation in a hanging drop, as a rule, have a very dense structure and mechanical strength [14], as well as spheroids obtained in microplates [10], which favorably distinguishes these approaches from our proposed one. At the same time, free-floating spheroids-conglomerates on low-adhesive substrates, are comparable or even more inferior in strength to the spheroids-clones obtained by us. The selection of cell cultures that secrete a large number of extracellular matrix molecules (collagen, laminin, hyaluronic acid, and others) is likely to improve the practice of obtaining spheroids in alginate drops with the density and strength characteristics necessary for the needs of bioprinting.

A characteristic feature of the spheroids obtained by us from the cells of both cultures was the absence of a central apoptosis/necrosis region, which is probably explained by their small size. It is known that the first signs of cellular damage under the influ-

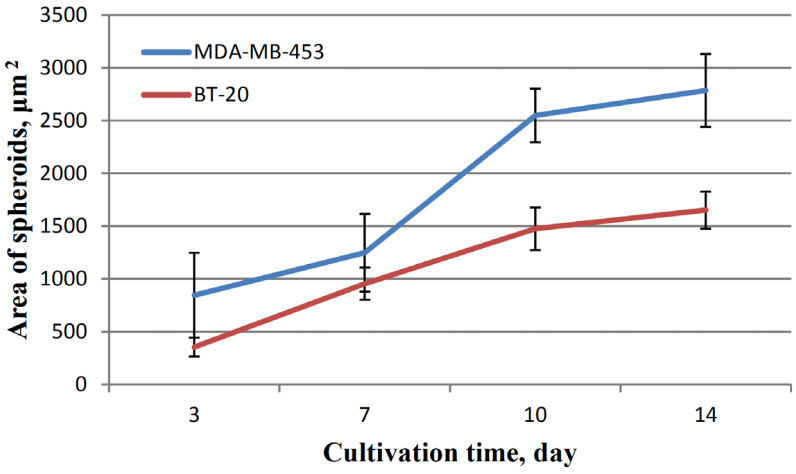

Fig. 3. Dynamics of growth of cellular spheroids formed in 2 % alginate gel by cells of BT-20 and MDA-MB-453 cultures. The data are presented as a sample mean value ± 95 % confidence interval of the general mean.

ence of hypoxia and lack of nutrients begin to manifest themselves in the center of the spheroids upon reaching their radius of 100 microns or more [15], in our case, the radius of even the largest cellular spheroids did not exceed 30 microns. Currently, it has been established that the adaptation of malignant cells and microenvironment cells to the lack of nutrients and oxygen observed in the tumor contributes to the development of chemo- and radioresistance, immunosuppression, invasion and metastasis, being one of the most important obstacles to cancer treatment [16]. In this regard, the reproduction of hypoxia and nutrient deficiency in a bioprinted cancer model has a special value. Large cellular spheroids are the main material on which hypoxia is studied *in vitro*, however, due to their size, bioprinting by extrusion through a thin nozzle with such structures is not possible. Therefore, in such models, direct imprinting of a suspension of cells into a biogel or a combination of methods of preforming sufficiently large cell conglomerates with subsequent pouring into a biogel is more often used [15]. Small spheroids, like those that we obtained by culturing cells of MDA-MB-453 and BT-20 cultures in alginate droplets, serve as good material for bioprinting, but do not show signs of hypoxia. Therefore, in this case, modeling of natural deficits existing in the tumor will be carried out not at the level of individual spheroids, but at the level of the entire model, where various gradients can be created by controlling the composition of the model components and their precise positioning relative to each other and sources of nutrients and gases.

In the MDA-MB-453 cell culture grown in alginate droplets, individual spheroids of different sizes with signs of degradation of cell nuclei were found (Figure 4b, isolation), indicating the beginning of cell death processes. This phenomenon, along with the uneven distribution in size and shape of the spheroids formed, may indicate a pronounced heterogeneity of clones formed by the MDA-MB-453 culture under these cultivation conditions. Heterogeneity is fundamentally unavoidable when it comes to spheroids-clones, and is a definite challenge for modeling tumor growth *in vitro* using such structures. In the case of multicellular conglomerates obtained by mechanical connection of cells (the hanging drop method or the formation of spheroids in cells), each such spheroid combines cells with different biological characteristics, as a result of which the difference between such cellular conglomerates becomes insignificant. Therefore, each multicellular spheroid can be considered an experimental repeat. In the case of clone spheroids, not every spheroid should be considered a separate experimental repeat, but their totality, which must be taken into account when designing a model of tumor growth that includes such structures. Thus, in order to obtain reliable results of the experiment of spheroid clones, tens of times more is required, which increases the cost of conducting research and thereby limits their scalability.

CONCLUSION

Using encapsulation in an alginate gel makes it possible to obtain in a short time a large number of cellular spheroids-clones suitable for bioprinting.

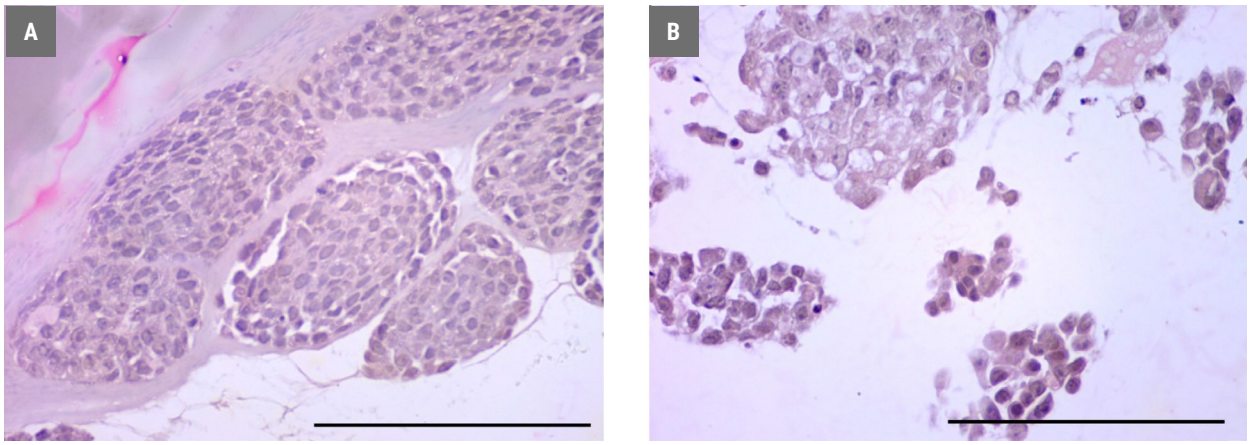

Fig. 4. Spheroids formed by cells of breast cancer cultures in a 2 % alginate gel on the 14th day of cultivation. a – culture BT-20, b – culture MDA-MB-453. Staining with hematoxylin-eosin.

The method we are testing is inferior to the methods of agglomeration of cells in a hanging drop and on microcells in terms of the speed of spheroid formation, as well as the density and uniformity of the resulting structures, but has an advantage over these methods in performance, which is comparable to the method of obtaining cellular spheroids by cultivation on low-adhesion substrates with constant mixing of the medium. The clonal nature of spheroids grown in alginate droplets somewhat increases the cost of conducting research using models derived from them compared to other approaches. However, such spheroids, in our opinion, are the best material for constructing, for example, bioprinted models of the development of micrometatstases, i.e. structures that by their nature are also clones of a single cell.

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CLINICAL CASE REPORT

NEW METHOD OF MODIFIED CHEMORADIOTHERAPY FOR CANCER OF THE UPPER AND MIDDLE AMPULLARY RECTUM

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ABSTRACT

The last decade is characterized by significant progress in the treatment of rectal cancer (reduction in the number of relapses to 5–6 % with the use of prolonged radiation therapy) before surgery. The greatest success has been achieved in the treatment of cancer of the lower ampulla of the rectum, when it is possible to develop a complete clinical response of the rectal tumor to chemoradiotherapy. Nevertheless, the requirement issues to improve the results of treatment of cancer of the upper and middle ampullar rectum with an increase in the survival of patients remain. Which makes it relevant to develop new methods, that increase the effectiveness of the treatment of rectal cancer.

The method of modified chemoradiotherapy for cancer of the upper ampulla of the rectum was developed in our study. The method is as follows: at the first stage, one day before the start of radiation therapy, the patient undergoes superselective catheterization of the superior rectal artery through the radial or femoral artery, followed by regional administration of radiomodifying chemotherapy drugs: cisplatin 50 mg and fluorouracil 500 mg. In one day, patients begin to undergo a course of conformal remote large-fraction radiation therapy to the primary focus and metastasis pathways for 5 sessions with a single focal dose of 5 Gy to a total focal dose of 25 Gy using a low-energy linear accelerator. During the entire course of radiation therapy, fluorouracil 500 mg is administered daily intravenously for 30 minutes in 30 minutes before the session. Surgical intervention with the sampling of material for research is carried out 6–8 weeks after the radiation therapy is completed. To assess the effectiveness of the modified chemoradiotherapy, the stage of tumor regression was determined according to the RECIST scale, and the level of therapeutic pathomorphology of the tumor according to Dworak was determined during a morphological study of the rectal tumor removed during the operation.

The developed method of modified chemoradiotherapy makes it possible to achieve regression of the rectal tumor in a short time, reduce the time and increase the effectiveness of treatment. The method of modified chemoradiotherapy is intended for patients with cancer of the upper and middle ampullar rectum T3-4N0-2M0, for whom radiation therapy is indicated as the first stage of treatment, after which resection of the rectum is performed in a standard volume.

Keywords: cancer of the upper and middle ampullary rectum, radiation therapy, radiomodification, chemotherapy, surgical treatment

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Compliance with ethical standards: the ethical principles presented by the Helsinki Declaration of the World Medical Association (World Medical Association Declaration of Helsinki, 1964, ed. 2013) were observed in the work. Informed consent was obtained from all participants of the study.

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

НОВЫЙ МЕТОД МОДИФИЦИРОВАННОГО ХИМИОЛУЧЕВОГО ЛЕЧЕНИЯ РАКА ВЕРХНЕ- И СРЕДНЕАМПУЛЯРНОГО ОТДЕЛА ПРЯМОЙ КИШКИ

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РЕЗЮМЕ

Последнее десятилетие характеризуется значительными успехами в лечении рака прямой кишки (снижение числа рецидивов до 5–6 % при применении пролонгированной лучевой терапии) перед оперативным вмешательством. Наибольший успех достигнут при лечении рака нижнеампулярного отдела прямой кишки, когда возможно развитие полного клинического ответа опухоли прямой кишки на химиолучевое лечение. При этом остаются проблемы необходимости улучшения результатов лечения рака верхне- и среднеампулярного отдела прямой кишки с увеличением выживаемости больных. Это делает актуальным разработку новых методов, повышающих эффективность лечения рака прямой кишки.
В нашем исследовании был разработан метод модифицированной химиолучевой терапии рака верхнеампулярного отдела прямой кишки. Метод заключается в следующем. Первым этапом за сутки до начала лучевой терапии пациенту выполняется суперселективная катетеризация верхней прямокишечной артерии через лучевую или бедренную артерию с последующим регионарным введением радиомодифицирующих химиопрепаратов: цисплатин 50 мг и фторурацил 500 мг. Через сутки больным начинает проводиться курс конформной дистанционной крупнофракционной лучевой терапии на первичный очаг и пути метастазирования в течение 5 сеансов с разовой очаговой дозой 5 Гр до суммарной очаговой дозы 25 Гр на низкоэнергетическом линейном ускорителе. На протяжении всего курса лучевой терапии больным за 30 мин до сеанса ежедневно внутривенно вводится фторурацил 500 мг в течение 30 мин. Хирургическое вмешательство с забором материала на исследование проводится через 6–8 недель после окончания лучевой терапии. Для оценки эффективности модифицированного химиолучевого лечения определяли степень регрессии опухоли по шкале RECIST, при морфологическом исследовании удаленной во время операции опухоли прямой кишки определяли степень лечебного патоморфоза опухоли по Dworak.
Разработанный метод модифицированной химиолучевой терапии позволяет добиться регрессии опухоли прямой кишки за короткий срок, сократить сроки и увеличить эффективность лечения. Метод модифицированной химиолучевой терапии предназначен для больных раком верхне- и среднеампулярного отдела прямой кишки T3-4N0-2M0, которым первым этапом лечения показана лучевая терапия, после чего выполняется резекция прямой кишки в стандартном объеме.

Ключевые слова: рак верхне- и среднеампулярного отдела прямой кишки, лучевая терапия, радиомодификация, химиотерапия, оперативное лечение

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Информированное согласие получено от всех участников исследования.

Финансирование: финансирование данной работы не проводилось.

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Substantiation

Treatment of rectal cancer depends on the stage of cancer, the localization of the tumor [1]. There are various treatment options for rectal cancer, which include surgery, radiation therapy, chemotherapy or a combination of these approaches [2–4]. The successful use of radiation therapy for malignant tumors of the rectum has led to the fact that it is included in the standards for the treatment of malignant tumors of this localization [5]. Methods of both preoperative and postoperative radiation therapy have been developed for the treatment of rectal cancer. However, preoperative radiation therapy proved to be the most effective. In many countries, including Russia, there is an increase in the frequency of use of preoperative radiation therapy and a decrease in the frequency of use of postoperative radiation therapy for rectal cancer. Thus, according to the study, in the USA from 2004 to 2011, in the structure of patients who received radiation therapy, the frequency of preoperative radiation therapy increased from 57 to 75 %, and the frequency of postoperative radiation therapy decreased from 39 to 18 % [6].

Radiation therapy for rectal cancer is possible in the form of a prolonged and short large-fraction course of radiation therapy. Numerous studies have been conducted to clarify the advantages and disadvantages of both methods of radiation therapy. The results of the studies have shown that patients with locally advanced malignant tumors of the rectum benefit from a prolonged course of radiation therapy, especially with lesions extending beyond the rectal wall and threatening to involve mesorectal fascia in the tumor process, as well as in cases of metastatic lesions of regional lymph nodes; with cancer of the lower ampullary rectum in order to increase the chances of performing sphincter-preserving operations and reducing the frequency of local relapses. A short course of radiation therapy with large dose fractionation is carried out with localized tumors of the upper and middle ampullary rectum, when preoperative therapy cannot change the volume of surgery on the rectum; if it is impossible to carry out a prolonged course of radiation therapy [7–9].

Conducting a preoperative course of prolonged radiation therapy reduces the 5-year recurrence rate in malignant tumors of the rectum by 2 times from 10.9 to 5.6 % [8; 10]. In the conducted randomized trials (SRCSG and SRCT), in addition to reducing the frequency of local relapses, an increase of 10 % in the overall and relapse-free survival of patients was also proven [11; 12].

A prolonged course of preoperative radiation therapy for distal rectal cancer may be so effective that it leads to a complete regression of the rectal tumor with the development of a complete clinical and pathomorphological response of the tumor to preoperative therapy [13–15]. As for cancer of the upper ampullary rectum, with this localization of the tumor, such a pronounced effect on neoadjuvant radiation therapy was not observed. All this indicates the need to develop and apply more intensive therapy regimens for the treatment of cancer of the upper ampullary rectum.

Purpose of the study is to develop a method of effective treatment of cancer of the upper ampullary rectum.

Research design

To increase the effectiveness of radiation therapy, we have developed a method of modified chemoradiotherapy, which allows us to achieve proper pathomorphological and therapeutic effects on the tumor, as well as to shorten the treatment time of patients. The method of modified chemoradiotherapy is intended for patients with cancer of the upper and middle ampullary rectum T3-4N0-2M0, who are shown radiation therapy as the first stage of treatment, after which rectal resection is performed in a standard volume.

Indications for the modified chemoradiotherapy were: unresectable locally advanced cancer of the upper and middle ampullary rectum T3-4N0-2M0; possibility of chemoradiotherapy; informed consent of the patient. Contraindications for the modified chemoradiotherapy were: unresectable rectal cancer, the presence of distant metastases, the impossibility of chemoradiotherapy.

Description of medical intervention

The method of modified chemoradiotherapy is as follows. The first stage, a day before the start of radiation therapy, the patient undergoes superselective catheterization of the upper rectal artery through the radial or femoral artery, followed by regional administration of radiomodifying chemotherapy drugs: cisplatin 50 mg and fluorouracil 500 mg. A day later, patients begin to receive a course of conformal remote radiation therapy for the primary tumor area and the area of regional metastasis, 5

sessions with a single dose of 5 Gy to a total focal dose of 25 Gy. During the entire course of radiation therapy, patients are injected daily intravenously with fluorouracil 500 mg for 30 minutes 30 minutes before the session. Surgical intervention on the rectum in the standard volume is performed 6–8 weeks after the completion of the course of radiation therapy. To assess the effectiveness of the treatment performed during the morphological examination of the surgical material, the stage of therapeutic pathomorphosis of the tumor is determined by Dworak.

Patient B., born on 09/20/1956, was admitted to the clinic of the National Medical Research Centre for Oncology on 02/03/2017 with complaints of blood and mucus in the feces, tenesmus, weakness.

It is known from the anamnesis that he considers himself ill since December 2016, when the above complaints appeared. I went to the doctor at my place of residence, a colonoscopy revealed a rectal tumor. Morphological analysis No. 449-58: G2 adenocarcinoma. The patient was sent to the National Medical Research Centre for Oncology, where a follow-up examination was conducted.

MRI of ACO and PO on 01/25/2017 revealed a tumor of the middle ampullary rectum with a spread to the upper ampullary 7.5 × 2.4 × 4.3 cm, at a distance of 8.1 cm from the anus, with lesion of mesorectal lymph nodes (Fig. 1a, b).

At the FCS on 12/28/2016, a rectal tumor was detected at a distance of 10 cm from the anus, examination to the caecum.

With a clinical diagnosis of cancer of the middle ampullary rectum cT3N1M0, ct 3B, cl.gr. 2, the patient was hospitalized for treatment.

On 02/07/2017, the patient underwent catheterization of the upper rectal artery through the femoral artery (Fig. 2), into which cisplatin 50 mg at 5 % glucose 50 ml and fluorouracil 500 mg were injected. On 02/08/2017, the patient began a short course of large-fraction radiation therapy (5 sessions of 5 Gy per primary tumor and regional lymph nodes), before the start of each session for radiation therapy, fluorouracil 500 mg was administered intravenously on a 0.9 % 200 ml sodium chloride solution for 30 minutes. After a 30-minute exposure, a radiation therapy session was performed. She underwent the course of modified chemoradiotherapy satisfactorily, there were no reactions and complications.

6 weeks after the end of the modified chemoradiotherapy, the patient underwent a control examination. An MRI of the abdominal cavity and pelvic organs on 03/20/2017 revealed a residual tumor of the middle ampullary rectum with signs of pathomorphosis, as well as a decrease in mesorectal lymph nodes in dynamics (fig. 3).

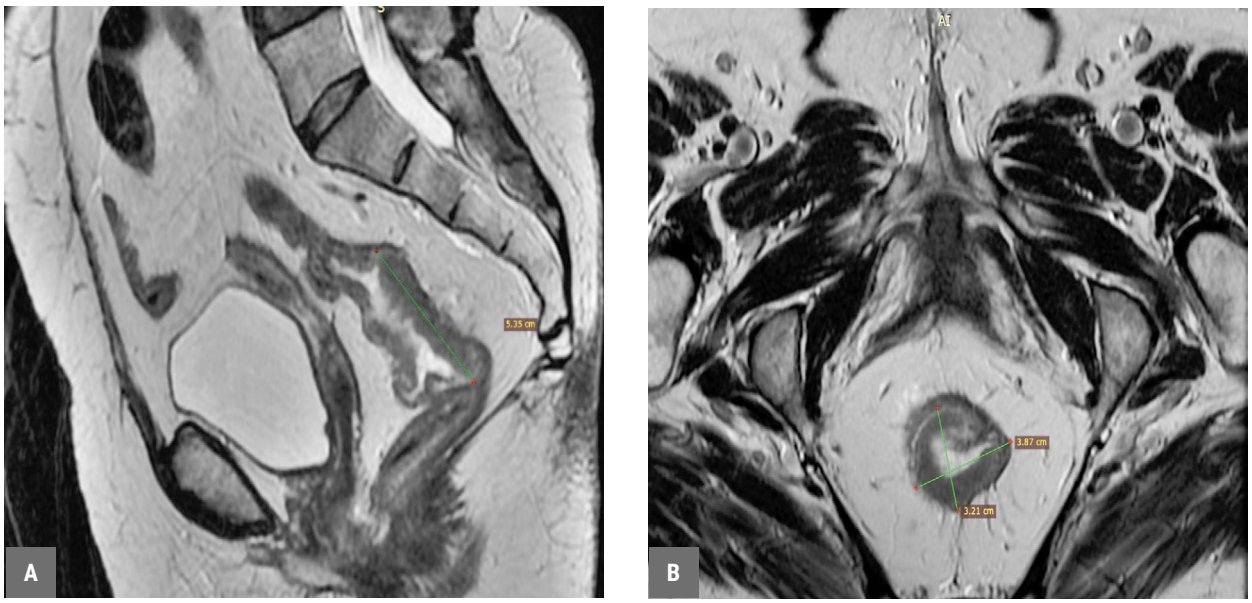

Fig. 1. MRI of the pelvic organs: a tumor of the middle ampullary rectum with a spread to the upper ampullary 7.5 × 2.4 × 4.3 cm, at a distance of 8.1 cm from the anus, with lesion of mesorectal lymph nodes (a) sagital section, (b) frontal section.

Rectoromanoscopy on 03/20/2017 revealed ulceration of the rectal mucosa up to 3 cm in diameter at 9 cm from the anus.

On 03/262017, the patient underwent surgery: anterior rectal resection with preventive ileostomy. Macropreparation: at the site of the tumor – ulceration of the mucous membrane of the rectum up to 3 cm in diameter (Fig. 4). Regional lymph nodes are not changed.

Histological examination of surgical material No. 24441-44/17: G2 adenocarcinoma with invasion of all layers of the wall, ulceration, inflammation. Signs of therapeutic pathomorphosis of the III grade – in the fibrous stroma of petrification; 24446-47/17 resection lines have the usual structure; 24445; 24448-49/17: in the lymph nodes sinus histiocytosis, focal lipomatosis.

Postoperative diagnosis: cancer of the middle ampullary rectum t3n1m0, st 3B, t.gr. 2, condition after chemoradiotherapy, T3N0M0. After surgery, she received courses of adjuvant chemotherapy with capecitabine. After 2 months, the patient completed the closure of the ileostomy without complications. The patient is observed without signs of progression to the present (more than 5 years).

Main study results

Thus, the given clinical example demonstrates a pronounced regression of the rectal tumor in a short period under the influence of modified radiation therapy: the tumor from 7.5 cm decreased to ulceration of the mucosa with a diameter of 3 cm (partial regression of the tumor on the RECIST scale) with the development of therapeutic pathomorphosis of the III grade.

Adverse effects

No adverse events were observed during the study.

DISCUSSION

Summary of the main research results

As a result of the study, a new method of modified chemoradiotherapy for cancer of the upper and middle ampullary rectum has been developed, which allows effective treatment in a short time with the development of therapeutic pathomorphosis of 3–4 stage.

Discussion of the main research results

The results of the application of the developed method of modified chemoradiotherapy have shown its effectiveness, which may be due to several mechanisms. Firstly, it is undoubtedly the modifying ef-

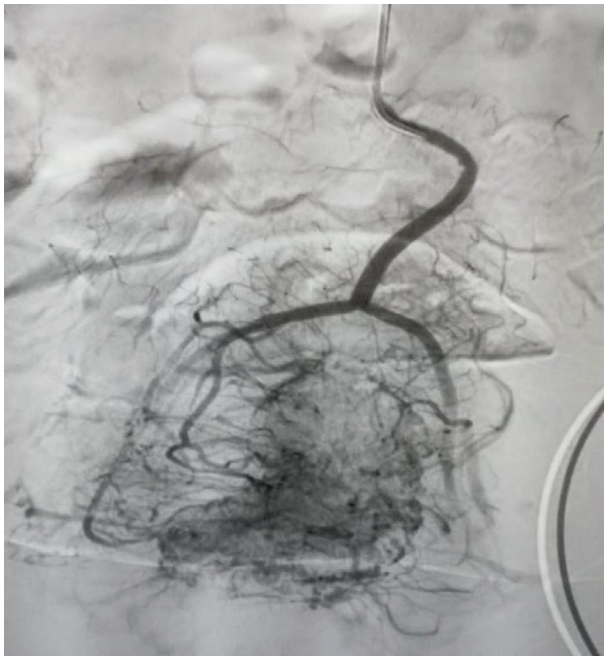

Fig. 2. Angiography: the upper rectal artery and its branches are contrasted.

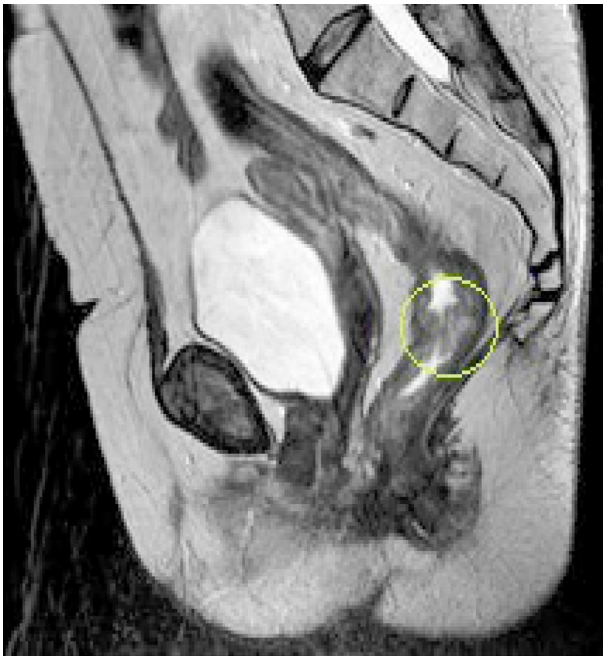

Fig. 3. MRI of the pelvic organs after modified chemoradiotherapy: residual tumor of the middle ampullary rectum with signs of pathomorphosis, as well as a decrease in mesorectal lymph nodes in dynamics.

fect of radiation therapy through the introduction of chemotherapy drugs. Secondly, this is the effect of regional chemotherapy, carried out superselectively through the upper rectal artery directly to the rectal tumor. The interaction of radiation and chemotherapy methods is also important. All these factors are involved in the development of therapeutic pathomorphosis of a rectal tumor of 3–4 stages in a short time, which reduces the duration of treatment of patients. Perhaps further movement in this direction will allow us to improve the method and achieve a complete clinical response of the tumor of the upper and middle ampullary rectum, as can be observed in cancer of the lower ampullary rectum.

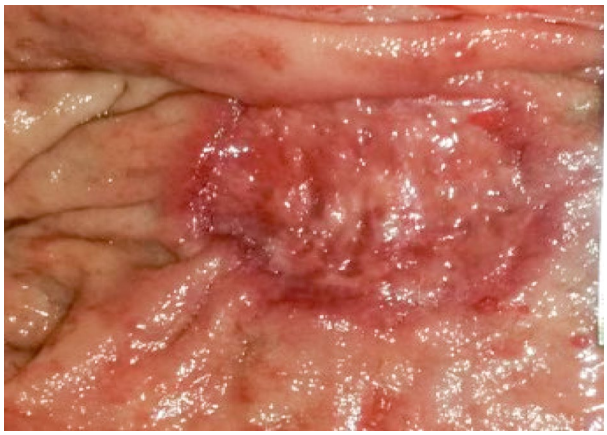

Fig. 4. Macropreparation: at the site of the tumor – ulceration of the mucous membrane of the rectum up to 3 cm in diameter.

CONCLUSION

So, a method of modified chemoradiotherapy for cancer of the upper and middle ampullary rectum was developed and put into practice, including preoperative radiation therapy with a short course of large dose fractionation and standard surgical treatment, characterized in that patients undergo superselective catheterization of the upper rectal artery before starting radiation therapy, followed by regional administration of radiomodifying chemotherapy drugs: cisplatin and fluorouracil. A day later, patients undergo a course of remote conformal large-fraction radiation therapy for the rectal tumor area and the area of regional metastasis, a total of 5 sessions with a single dose of 5 Gy to a total dose of 25 Gy. During the entire course of radiation therapy, patients are injected daily intravenously with fluorouracil 500 mg for 30 minutes 30 minutes before the session. Surgical intervention on the rectum in the standard volume is performed 6–8 weeks after the completion of the course of radiation therapy. The use of the modified chemoradiotherapy method allows for highly effective treatment for cancer of the upper and middle ampullary rectum, confirmed by the development of therapeutic tumor pathomorphosis. These data indicate the effectiveness of the developed method of modified chemoradiotherapy for rectal cancer, which makes it possible to recommend it for use in clinical practice.

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CLINICAL CASE REPORT

SUCCESSFUL EXTIRPATION OF A PERFORATED ESOPHAGUS AFTER CHEMORADIOTHERAPY IN INFILTRATIVE ULCERATIVE SQUAMOUS CELL CARCINOMA

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ABSTRACT

Esophageal cancer is one of the most aggressive malignant neoplasms of the gastrointestinal tract, occupying the eighth place in the structure of morbidity worldwide. Despite comprehensive approaches to treatment, mortality continues to grow in both gender groups, which moves this pathology to the sixth position in the structure of mortality from malignant tumors. A lot of patients undergo radiation therapy in the preoperative period or in an independent version due to the peculiarities of the localization of the tumor or the spread of the process. One of the serious complications of the disease on the background of ongoing conservative therapy is perforation of the esophagus, which, according to the literature, can develop from 5.6 to 33 % of cases, and the risk factors for the development of this complication are infiltrative-ulcerative form of cancer, disease stage T3–4 and the presence of esophageal stenosis, as well as the use of chemotherapy drugs such as fluorouracil and cisplatin. The article describes a clinical case of esophageal perforation in a patient with infiltrative-ulcerative form of squamous cell carcinoma of the esophagus on the background of preoperative chemoradiotherapy. The total focal dose (TFD) at the time of complication development was 24 Gy. As a result of a comprehensive additional examination, which revealed a developed complication in the form of perforation of the esophagus, an interdisciplinary council decided on an immediate surgical intervention, during which extirpation of the esophagus with gastro- and esophagostomy was performed. The patient was discharged on the 15th day in a satisfactory condition with a recommendation to conduct an IHC study for the presence of PD-L1 expression to determine further management tactics. This clinical case demonstrates the role of the infiltrative-ulcerative form of tumor growth, the stage of the disease, as well as the use of chemotherapy drugs during radiation treatment as risk factors for the development of esophageal perforation; an important task at the prehospital stage in the selection of such patients is a thorough examination in specialized oncological centers to exclude possible complications in the process of the above conservative treatment.

Keywords: chemoradiation therapy, esophageal cancer, esophageal perforation

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КЛИНИЧЕСКОЕ НАБЛЮДЕНИЕ

УСПЕШНАЯ ЭКСТИРПАЦИЯ ПЕРФОРИРОВАННОГО ПИЩЕВОДА ПОСЛЕ ХИМИОЛУЧЕВОЙ ТЕРАПИИ ПРИ ИНФИЛЬТРАТИВНО-ЯЗВЕННОМ ПЛОСКОКЛЕТОЧНОМ РАКЕ

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РЕЗЮМЕ

Рак пищевода – одно из самых агрессивных злокачественных новообразований (ЗНО) желудочно-кишечного тракта, занимающее восьмое место в структуре заболеваемости во всем мире. Несмотря на комплексные подходы к лечению, смертность продолжает расти в обеих гендерных группах, что перемещает данную патологию на шестую позицию в структуре смертности от ЗНО. Множество пациентов проходят лучевую терапию в предоперационном периоде или в самостоятельном варианте в силу особенностей локализации опухоли или распространенности процесса. Одним из серьезных осложнений заболевания на фоне проводимой консервативной терапии является перфорация пищевода, которая, по литературным данным, может развиваться от 5,6 до 33 % случаев, а факторами риска развития данного осложнения являются инфильтративно-язвенная форма рака, стадия заболевания T_{3-4} и наличие стеноза пищевода, а также применение таких химиопрепаратов, как фторурацил и цисплатин. В статье описан клинический случай развития перфорации пищевода у пациента с инфильтративно-язвенной формой плоскоклеточного рака пищевода на фоне проводимого предоперационного химиолучевого лечения. Суммарная очаговая доза (СОД) на момент развития осложнения составила 24 Гр. Вследствие комплексного дообследования, выявившего развившееся осложнение в виде перфорации пищевода междисциплинарным консилиумом было принято решение о немедленном хирургическом вмешательстве, в ходе которого была выполнена экстирпация пищевода с гастро- и эзофагостомией. Пациент был выписан на 15-е сутки в удовлетворительном состоянии с рекомендацией проведения ИГХ-исследования на наличие экспрессии PD-L1 для определения дальнейшей тактики лечения. Данный клинический случай демонстрирует роль инфильтративно-язвенной формы роста опухоли, стадии заболевания, а также применение химиопрепаратов во время лучевого лечения как факторы риска развития перфорации пищевода; важной задачей на догоспитальном этапе при отборе таких пациентов служит тщательное обследование в специализированных онкологических центрах для исключения возможных осложнений в процессе вышеописанного консервативного лечения.

Ключевые слова: химиолучевая терапия, рак пищевода, перфорация пищевода

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Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Информированное согласие получено от всех участников исследования.

Финансирование: финансирование данной работы не проводилось.

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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RELEVANCE

Esophageal cancer is one of the most aggressive malignant neoplasms of the gastrointestinal tract, occupying the eighth place in the structure of morbidity worldwide [1]. Despite comprehensive approaches to treatment, mortality continues to grow in both gender groups [2], which places this pathology to the sixth position in the structure of mortality from MNs [1].

Surgical intervention may be delayed or even impossible due to the features of the localization of the tumor or the prevalence of the process [3; 4], therefore, many patients undergo complex treatment, including radiation therapy, in the preoperative period or in an independent version.

One of the serious, high mortality rate complications of the disease on the background of conservative therapy is perforation of the esophagus. The causes of esophageal perforations are multiple, the main factors are iatrogenic effects and mechanical damage (barogenic ruptures, damage by foreign bodies, blunt chest injuries). According to a number of authors [5–7], the formation of an esophageal fistula as a complication of radiation therapy can develop in 5.6 to 33 % of cases, therefore, patients with a widespread tumor process require a thorough examination before starting treatment.

Purpose of the study: to present the results of clinical observation of a patient who developed a complication in the form of esophageal perforation at the stage of the preoperative course of chemoradiotherapy.

CLINICAL CASE INTRODUCTION

Patient B., 55 years old, was admitted to the radiotherapy Department No. 2 at the National Medical Research Centre for Oncology, Rostov-on-Don. The diagnosis was: (C15) Cancer of the middle and lower third of the esophagus T3N2M0, III stage, cl. gr. 2, with chronic bronchitis as concomitant pathology. From anamnesis: ill for 1 month, worried about weakness and increasing dysphagia. During fibrogastroduodenoscopy (FGDS) at the place of residence, an infiltrative ulcerative tumor of the esophagus was detected, a biopsy of the formation was performed. A histological examination of the biopsy material was carried out, conclusion No. 13420-21 of 07/27/2022: low-grade cancer. Independently applied to the National Medical Research Centre for Oncology, for further examination and determination of treatment tactics. When reviewing histological preparations at the National Medical Research Centre for Oncology No. 2011/22 dated 08/10/2022, fragments of the esophageal mucosa with complexes of low-grade carcinoma were determined. For the purpose of differential diagnosis between squamous cell carcinoma without keratinization and adenocarcinoma, an IHC study was recommended. 08/10/2022 a CT scan of the chest, abdominal cavity and pelvis was performed, conclusion: tumor of the middle and lower third of the esophagus for 6.7 cm with thickening of the walls to 1.6 cm with infiltration of the surrounding fiber, metastatic lesion of the intra-thoracic, retroperitoneal, subclavian lymph nodes (Fig. 1). Conclusion of immunohistochemical study No. 2524/22 dated 08/22/2022: The morphological picture and immunophenotype of tumor cells in the volume of esophageal biopsy (SC5/6+) are most characteristic of low-grade squamous cell carcinoma. Based on the data obtained, a consultation of physicians at the National Medical Research Centre for Oncology recommended a course of chemoradiotherapy as the first stage of treatment. Objectively upon admission: the general condition is close to satisfactory. Complaints of general weakness. The skin is of ordinary color, clean. There is vesicular respiration above the pulmonary fields, with no wheezing identified. Heart tones are muted, rhythmic, blood pressure of 121/78 mmHg, 76 beats/min heart rate. Body temperature 36.5 °C. The abdomen is soft, painless on palpation, the liver is at the edge of the costal arch, the spleen is not palpable. Healthy bladder and bowel habits. Weight of 80 kg, height of 180 cm, body area about 2 m^2.

Since 08/23/2022, a course of remote chemoradiotherapy on a linear accelerator Truebeam (Varian, USA) has been started using the technology of volume-modulated arc therapy (VMAT) for the esophageal tumor area and regional lymphatic collector with a single focal dose (SFD) of 2 Gy to a total focal dose (TFD) of 50 Gy for 25 fractions. No abnormalities were detected during the control of hematological parameters. On 08/25/2022, chemotherapy drugs were administered against the background of infusion therapy according to the scheme: paclitaxel 50 mg/m^2 (100 mg) and car-

boplatin AUC2 (360 mg). On 09/01/2022 the 2nd administration of drugs in the previous dose was carried out. 09/07/2022 after the 12th session with TFD 24 Gy, the patient complained of pain in the chest area on the right, in connection with which he was immediately examined to exclude cardiac pathology. On the ECG from 09/07/2022, a sinus rhythm with a heart rate of 70 beats/min, frequent supraventricular extrasystole episodes by the type of bigeminia, violation of intraventricular conduction, signs of left ventricular myocardial hypertrophy are recorded. An hour after the appearance of chest pain, complaints of shortness of breath joined. During the spiral computed tomography (CT) scan of the chest organs, signs of perforation of the esophagus with the formation of a right-sided hydropneumothorax were revealed (Fig. 2). The patient was urgently taken to the operating room for life-threatening indications. Intraoperatively: the patient is laid on his left side in the position for anterolateral thoracotomy on the right in the V intercostal space. Under endotracheal anesthesia with artificial ventilation, a thoracotomy was performed in the V intercostal space on the right. During revision up to 200 ml of serous-purulent effusion with digestive contents in the pleural cavity. In the mid-thoracic part of the esophagus, a tumor with necrotic changes was palpated, the latter up to 5 cm in size with a transition to the visceral pleura of the middle lobe of the lung. In the center of necrosis there is a perforating hole up to 1.5 cm in diameter. Tumor-necrotic-inflammatory infiltrate involved v.azygos and tributaries. With technical difficulties due to localization and prevalence of the process, v.azygos and tributaries were mobilized, bandaged twice, crossed. Mobilization of the esophagus with mediastinal lymphadenectomy was performed, necrectomy of the visceral pleura of the middle lobe of the lung was performed, areas suspected of leakiness (with an aqueous sample) were sutured with monolithic sutures, the pleural cavity was drained with 2 PVC tubes installed along the posterior axillary line in the 7th and 8th intercostals on the right. The patient was turned over to a position on his back. The cervical esophagus was decorated on the neck in the form of an esophagostomy. Upper median laparotomy was performed. The abdominal part of the esophagus is extracted into the abdominal cavity. The diaphragm legs are sutured. An additional row of stitches was applied to the stump of the esophagus. A gastrostomy was applied for nutrition – it was removed through a separate puncture, and the laparotomy wound was sutured in layers, tightly.

After surgery, the patient stayed in the anesthesiology and intensive care unit for 8 days. Further, in the specialized department he received treatment, including: infusion therapy (glucose-electrolyte and colloidal solutions), antibacterial therapy, antispasmodics, analgesics, prevention of postoperative pancreatitis, thromboembolic complications, metabolic and restorative therapy, inhalations, regular dressings. Drains from the abdominal and pleural cavities have been removed. Postoperative wound – healing by primary tension in suture conditions. The gastrostomy is functioning.

The patient was discharged on the 15th day in a satisfactory condition with a recommendation to conduct an IHC study for the presence of PD-L1 expression to determine further treatment tactics.

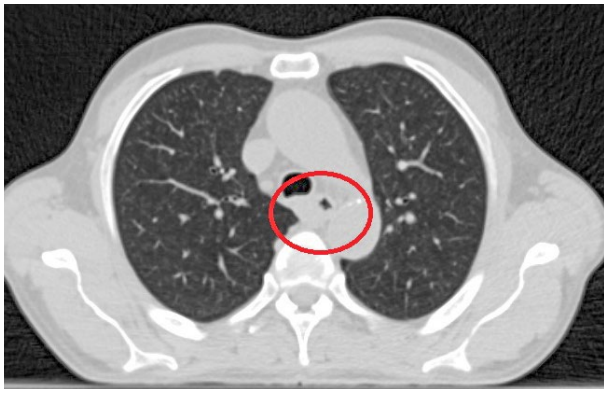

Fig. 1. Spiral chest CT before treatment.

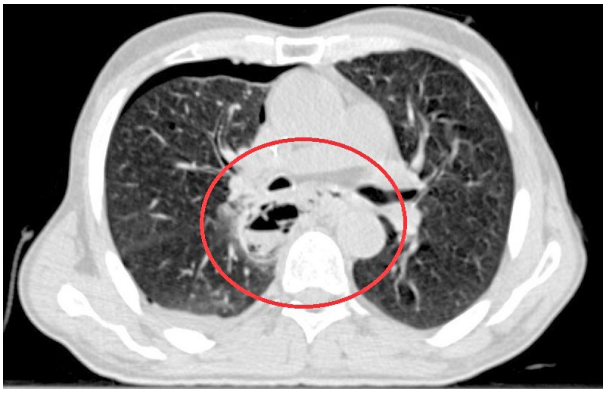

Fig. 2. Perforation of the esophagus with the formation of a hydropneumothorax on the background of radiation therapy course.

DISCUSSION

Zhu C et al. [5] analyzed 78 studies conducted in between 1990 and 2018. They included 1,866 patients with an established diagnosis of esophageal cancer receiving radiation treatment (chemoradiotherapy, brachytherapy or radiation therapy in an independent version). Esophageal perforation was detected in 200 cases and amounted to 10.7 %. The authors found out that patients younger than 60–65 years were at risk; no statistically significant results were obtained regarding gender. In patients with ulcerative form of the tumor, stage T3–4 and squamous histological structure of cancer, perforation also developed more often. Esophageal stenosis has also been described as a risk factor. When analyzing studies describing the results of chemoradiotherapy in various modes, it was found that the complication developed more often against the background of the use of fluorouracil and cisplatin, in contrast to treatment with a combination of taxanes and cisplatin.

A retrospective analysis of risk factors for esophageal perforation against cancer, conducted by Hai-yan Chen et al. [8], included 322 patients who underwent radiation therapy due to the unresectability of the tumor, or relapse of the disease. The complication occurred in 10 patients during radiation therapy, and 8–40 weeks after completion in 8 more patients, which totaled 5.8 %. All the patients were male. 14 of the 18 patients (77.7 %) were under 60 years of age. 12 patients (66.6 %) were treated with stage T4. Chemoradiotherapy was received by 9 people (50 %). The average radiation dose at which perforation occurred was 54 Gy. Based on the above data, the authors concluded that the risk of esophageal perforation is male, age under 60 years and stage T4. The dependence of the complication development relative to the histological type has not been described.

Bing Hu et al. [9] analyzed 414 cases of chemoradiotherapy of squamous cell carcinoma of the esophagus in various regimens from 2012 to 2018 and found that esophageal perforation occurred in 46 patients (11.1 %), among whom 40 patients were men (86.96 %); 20 of them (43.48 %) were under the age of 60. 27 patients (58.7 %) were treated with stage T4. All patients with the developed complication were diagnosed with esophageal stenosis of varying degrees.

Based on the above research results, as well as our own experience, we can identify such risk factors for the development of esophageal perforation as an infiltrative-ulcerative form of the tumor, stages of the disease T3-4, esophageal stenosis and the use of fluorouracil and cisplatin drugs.

CONCLUSION

Taking into account the improvement of technical equipment, the possibilities of selecting patients for the provision of neoadjuvant radiation therapy of malignant neoplasm (MN) have expanded. It is necessary to remember and take into account the role of the infiltrative-ulcerative form of tumor growth as a risk factor for the development of esophageal perforation in esophageal cancer with squamous cell histotype. An important goal in the selection of such patients is a thorough examination in specialized cancer centers to exclude possible complications during the conservative treatment process.

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