ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2012. Випуск 60. С. 137–144
Visnyk of the Lviv University. Series BIology. 2012. Issue 60. P. 137–144

UDC 616-006.04-07

CLINICAL SIGNIFICANCE OF USING METHOD OF FLUORESCENT PROBES AND METHOD OF WEDGE DEHYDRATION IN ASSESSING ALBUMIN PARAMETERS IN CANCER PATIENTS

N. Lukouskaya[1], A. Shaforost[1], A. Malenchenko[1], V. Belyakovskiy[2], D. Okuntsov[2], T. Prigozhaya[2], S. Stasenkova[2], N. Krutilina[2], L. Parkhomenko[2]

[1]Institute of Radiobiology of NAS of Belarus
4, Feduninskyi St., Gomel 246007, Belarus
e-mail: irb@mail.gomel.by
[2]Gomel Regional Clinical Oncology Center
2, Medical St., Gomel 246012, Belarus
e-mail: ic@grcoc.gomel.by

It is shown that the total albumin concentration in blood serum of healthy people and cancer patients is within normal range. A statistically significant change in parameters «effective albumin concentration» and «index of toxicity» that reflects the efficiency of radiation therapy, involving a process of destruction of tumor cells and an intake products of catabolism in the bloodstream. These results agree with data obtained by the method of wedge dehydration, which can be used to visualize the status of biological fluids and the impairment of their properties during pathological processes.

Keywords: albumin, cervical cancer, head and neck cancer, TAC, EAC, RAB, IT, method of wedge dehydration

Introduction. Most diseases and pathological conditions, including radiation damage of the body, develop against syndromes of exogenous or endogenous intoxication, accompanied by the accumulation of toxic compounds in excessive concentrations or forms that are not typical for normal metabolism. Major systems of the binding and transport of substances of different chemical nature in the body are immune antibodies, buffer system, blood cells, and plasma proteins. Dominant among these is the serum albumin.

Serum albumin is the main extracellular transport system, accounting for about 70% of plasma proteins. Molecule of albumin has a high affinity to many endogenous and exogenous physiologically active compounds, such as bilirubin, fatty acids, hemin, porphyrin derivatives, steroid hormones, heavy metals, etc., as well as a variety of xenobiotics − drug compounds and poisons. It should also be noted that the antioxidant activity of blood plasma to a large extent due to albumin. As a non-specific component of the antioxidant system serum albumin is highly effective in neutralizing free radicals. The antioxidant properties of serum albumin predetermine its properties under stress and pathological processes in the body [1].

Albumin, as a component of body fluid is present in all tissues and is an active member of the metabolic processes. About a third of its weight (120 g) is in the blood, the rest (300 g) is outside the bloodstream. Blood and tissue pools of albumin are exchanged and, therefore, blood albumin is an informative indicator of the metabolic processes of the body. Activity of such protein is determined by the conformational state of the molecule [4]. Under the influence of various physical and chemical factors in the complexation process there is a change of the spatial structure of the molecule, which is reflected in the functional activity of the protein, in the first place, the availability of binding sites [1, 4].

Institute of Physico-Chemical Medicine (Russian Federation) proposed fluorescence method for determining the state of binding sites of serum albumin, allowing to define such parameters as «total albumin concentration» and «effective albumin concentration» [1]. Based on published data [2–4, 6–8, 11, 13, 14], the definition of albumin parameters allows to estimate not only the number of molecules of serum albumin, but also their structural and functional changes in all phases of the diagnostic and therapeutic process in patients. However, in clinical practice, analysis of albumin parameters is not widely used.

V. N. Shabalin and S. N. Shatokhina developed a method of wedge dehydration, which allows to obtain information on the general status of various biological fluids using the morphological picture of a dried droplet (facies) [15, 16].

Biocolloids – serum of blood, is capable of spatial self-organization. The vast majority of protein molecules in the serum of blood are in the form of supramolecular complexes. Organized autowave structure of dehydrated drop reflects the state of these complexes. During the drying droplet of biological fluids occurs redistribution of organic and inorganic components of the drop followed by cracking of the surface: formed facies with radial and cellular structure. It has three clearly differentiated areas: central, peripheral and transitional. Formed static order is optically detected and gives information about the molecular organization bioliquid at the macroscopic level [12, 15, 16].

The purpose of this work – using the method of fluorescent probes and the method of wedge dehydration to study structural and functional state of human serum albumin of patients with cervical cancer, head and neck cancer during radiation therapy.

Materials and methods

We studied serum albumin of 47 people: 13 healthy (control); 11 patients with cervical cancer undergoing a split course of radiation therapy (first stage – 30 Gy, with a break of three weeks the second stage – 20 Gy); 4 patients with the same diagnosis, undergoing an unsplit radiotherapy (40 Gy); 19 patients with head and neck cancer undergoing an unsplit radiation therapy (70 Gy).

State of binding sites of serum albumin was estimated by the method of Y.A. Gryzunov et al. [1]:

- Total Albumin Concentration (TAC) – the number of albumin molecules;
- Effective Albumin Concentration (EAC) – the number of unoccupied binding sites of albumin;
- Reserve Albumin Binding (RAB=EAC/TAC·100%) – reflects the degree of structural modification of the protein;
- Index of Toxicity (IT=TAC/EAC-1) – characterizes the filling of albumin centers by toxic ligands.

For optimum accuracy of the analysis temperature of the samples during the measurements was + 23±2°C.

This method is based on the specific binding of the fluorescent probe K-35 and albumin in serum of blood. Probe was synthesized by B. M. Krasovitskiy, L. I. Kormilovoy and I. G. Ermolenko (Harkov), contains a carboxyl group, which at pH 7.4 carries a negative charge [1, 7].

D. E. Dobretsov and Y. I. Miller [1, 7] shows that the molecules of probe – organic anion, has fluorescence maximum at in 515 nm. If serum of blood is divided on fractions, that the probe in the lipoprotein fraction fluoresces very weakly, as in the buffer solution without protein. Significant fluorescence is observed only in the albumin fraction. Adding globulin to albumin fraction does not affect the fluorescence. Thus, virtually all fluorescent signal of K-35 in the serum of blood due to the molecules of probe bound to albumin. About 23% the molecules of K-35 in albumin associated with the centers, where the water can not access the probe. Centers of type 1 and 2 account for about 50% of the total fluorescence of the probe. Consequently, the fluorescence of K-35 is equally responsive to the presence in albumin metabolites, which fill both centers.

It should be noted there is a decrease of binding of the probe K-35 with albumin in the serum of patients, the reasons for this are:

* competition of dye and other low-molecular ligands, mostly hydrophobic, for binding sites in albumin;

* interaction albumin with high-molecular components of plasma and impairment of «adaptation» of centers of albumin to ligands.

To determine the total albumin concentration the research are carried out at pH=4.2 and in the presence of nonionic detergent bridge-35. The shift of pH to the acid side induces a conformational transition N-F of the molecule of albumin. In this case, there is a decrease and alignment (in value) of binding constants of various ligands to albumin, as well as «opening» of the binding sites of molecule by increasing its ellepticity. Thus, binding of interfering hydrophobic ligands to albumin is weak. Bridge-35 the following functions: its micelles serve as «buffer capacity» to probe K-35 and competitively fulfils (in relation to albumin) bind hydrophobic ligands; detergent solubilizes the high-molecular components of serum of blood, thus, preventing their interaction with albumin. As a result of these actions of both pH, and detergent the binding sites of albumin are mostly free, and the conformation of the protein − stabilized.

At physiological pH the fluorescence intensity of the probe K-35, upon interaction with serum albumin, depends not only on the concentration of the protein, but also on the physical and chemical state of its globules: the presence of ligands (metabolites, toxins), covalent and non-covalent modification of amino acid residues, the conformation − that is, factors, which vary depending on the condition of the body. This fluorescence intensity is expressed in units of concentration (g/l) and is called «effective albumin concentration».

Besides investigating using fluorescence method in the blood of examined patients was analyzed the state of the serum of blood by method of wedge dehydration. Initially, blood samples were centrifuged (3 min at 3000 vpm), and then 10 µl of serum was applied to a glass slide, skim alcohol. To verify the identity of paintings of dried droplets (facies) produced 2–3 reps. Drying was carried out for 24 hours at room temperature. Facies were studied using an optical microscope in transmitted and reflected light [15, 16].

Analysis of the data was performed using GraphPad Prizm 4.0. Statistical processing is made using t-test at a significance level $P<0.05$ and $P<0.01$. Data are presented as the mean ± standard deviation (M±m).

Results and discussion

In the course of the study it was found that TAC in healthy people and patients with head and neck cancer, cervical cancer at admission to hospital was within the normal range (Table 1–2).

In the analysis of the EAC in healthy this index was 46.68 g/l and was significantly decreased to 29.24 g/l in patients with head and neck cancer; in patients with cervical cancer was 35.87 g/l (at admission to a split course of treatment) and 33.40 g/l (at admission to an unsplit course of treatment). Index of IT was 0.05 in healthy; in patients with head and neck cancer 0.68; in gynecological cancer patients − 0.48 (at admission to a split treatment) and 0.55 (at admission to an unsplit treatment).

In healthy people, mainly albumin binding sites are free from toxic ligands, so EAC is close to TAC. Consequently, IT is close to zero. At pathological processes of various origins EAC becomes lower TAC owing to filling centers of albumin by toxins and increases in the value of IT.

Value of RAB in healthy people is 95.06%; in patients − 61.34% in head and neck cancer and 71.02% in cervical cancer (at admission to a split treatment) and 64.80% (at admission to an unsplit treatment).

In the middle of a treatment course (40–50 Gy) in patients with head and neck cancer observed the 2nd stage of the radiation reactions and damage on a scale EORTC/RTOG evaluation of acute radiation reactions, and values of IT decreased on 14.7% as compared to patients at admission to treatment.

Upon completion of the treatment of patients with cervical cancer undergoing a split course of radiation therapy the values of parameters were as follows: TAC significantly increased to 55.48 g/l; the EAC has remained virtually unchanged and at the end of the 2nd stage was 34.39 g/l; IT=0.72 after the 1st stage of treatment, but after the 2nd increased from 0.56 to 0.66. In patients who undergoing an unsplit course of therapy: TAC had no significant difference before and after radiation therapy; EAC=33.25 g/l; IT has increased on 56.3% and become 0.86.

Perhaps change the values of IT (0.66 and 0.86) in patients with cervical cancer undergoing a course of radiotherapy in different modes, due to the fact that during a three-week break between the two stages of the split course the available toxins eliminated from the body. The latter follows from the data: IT=0.72 after the 1st stage of the split course and IT=0.56 at admission to the 2nd stage.

In patients with head and neck cancer at the end of radiation therapy the values of indexes were as follows: EAC=30.78 g/l; the values of TAC was not significantly different before and after treatment; RAB was 68.39%; IT down to 0.52.

Table 1

Indicators TAC, EAC, RAB and IT in the serum of blood of healthy people and patients with head and neck cancer undergoing the radiation therapy

Index	Healthy people (n=13)	Patients with head and neck cancer (n=19)		
		admission	middle course	hospital discharge
TAC, g/l	49.21±1.37	47.84±2.10	45.05±1.82	44.92±1.73
EAC, g/l	46.68±1.19	29.24±1.69**	29.36±1.75**	30.78±1.73**
RAB, %	95.06±1.27	61.34±2.61**	64.73±2.39**	68.39±3.24**
IT, rel. units	0.05±0.01	0.68±0.07**	0.58±0.05**	0.52±0.07**

Comment. The reliability of indices when compared to control: * − P<0.05; ** − P<0.01

Table 2

Indicators TAC, EAC, RAB and IT in the serum of blood of healthy people and patients with cervical cancer undergoing radiation therapy

Index	Healthy people (n=13)	Gynecological cancer patients undergoing a split course of radiotherapy (n=11)				Gynecological cancer patients undergoing an unsplit course of radiotherapy (n=4)	
		admission to the 1st stage	hospital discharge	admission to the 2nd stage	hospital discharge	admission	hospital discharge
TAC, g/l	49.21±1.37	50.63±2.17	49.11±3.00	50.05±2.16	55.48±2.51*	51.80±5.55	55.57±4.59
EAC, g/l	46.68±1.19	35.87±2.77**	30.44±3.00**	32.29±1.36**	34.39±1.97**	33.40±3.28**	33.25±8.14*
RAB, %	95.06±1.27	71.02±4.89**	60.91±4.03**	65.23±2.88**	62.84±3.98**	64.80±2.81**	58.10±9.62*
IT, rel. units	0.05±0.01	0.48±0.10**	0.72±0.13**	0.56±0.07**	0.66±0.14*	0.55±0.06**	0.86±0.29**

Comment. The reliability of indices when compared to control: * − P<0.05; ** − P<0.01

Thus, from the obtained data the values of IT at the end of radiation therapy are controversial: an increase IT in patients with cervical cancer and lower IT in patients with head and neck cancer.

Perhaps the dynamic changes of IT after radiation therapy in patients with different tumor localization related to the complex influence of the following factors:

1. *Distinctive modes of radiation therapy:* 1st group of patients with cervical cancer undergoing a split course of radiation therapy (1st stage – 30 Gy, with an interval of 3 weeks, 2nd stage – 20 Gy), 2nd group of patients with cervical cancer undergoing an unsplit radiotherapy (40 Gy), 3rd group of patients with head and neck cancer undergoing unsplit course of radiotherapy (70 Gy).
2. *Individual radiosensitivity of patients to the action of the radiation factor (a course of radiation therapy):* patients have different rate of occurrence of the metabolic processes and the destruction of tumor cells and their subsequent elimination.
3. *Radiotoxins action:* the concept of radiotoxins as substances are produced in the body by radiation, which are biologically active, and causing radiation effects in the irradiated organism, dated back to the early days of radiobiology. As radiotoxins may be: quinones, unsaturated fatty acids, organic and inorganic peroxides, protein decomposition products, choline, histamine, protein antigens, various metabolites and cytotoxic agents unexplored nature. It is known that at radiation therapy observed treatment effect will be due to cell death, which occurs under the influence of the direct action of high-energy particles on the unique cellular structure, and as a result of secondary processes, including the action occurring in the tumor tissue radiotoxins which will play a significant role in attacking the unique structure, preventing their repair [5, 9].

Primary importance in the transmission of the radiation influence is blood which in the irradiated organism acquires new biological properties: contains various radiotoxins. As mentioned above, the basic system of binding and transport of substances of different chemical nature, including radiotoxins is serum albumin. Thus, dynamic change of values of IT, characterizing the filling of albumin centers by toxic ligands, illustrates the individual characteristics of the transport system of serum albumin of cancer patients.

Structural and functional changes of albumin in cancer identified by fluorimetry, are consistent with data obtained by the method of wedge dehydration. It is known that facies of healthy people have a pronounced right radial structure of the cracks and the lack of any additional structures (Fig. 1) [10].

Figures 2 and 3 show the changes of the morphological picture of serum of blood of patients with cervical cancer at admission to treatment, and after a course of radiotherapy. In the initial stages of treatment there is a tendency to wors morphological picture of serum albumin, resulting in a violation of the radial symmetry of facies and appearance of pathological formations. By the end of treatment the morphological picture of serum of blood has a broken radial symmetry. This fact can be explained by the presence in the blood of the decay products of tumor cells (radiotoxins) formed during the course of radiotherapy. This is confirmed by an increase of IT at the time of discharge of patients.

Study of the reaction of the organism to radiation during the full course of radiation therapy in patients with head and neck cancer revealed distorted morphology of facies from admission (Fig. 4) to the middle of the course (Fig. 5), which also can be associated with the arrival of the decay products of tumor cells to blood and the presence of formed radiotoxins. At the moment of discharge of patients there is an improvement of the morphological picture of facies (Fig. 6), manifested in the sequence and reconstruction of the radial structure.

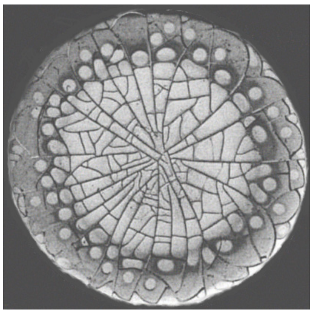

Fig. 1. Facies of serum of healthy human.

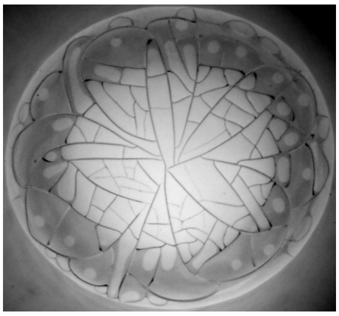

Fig. 2. Facies of serum of patient with cervical cancer at admission to treatment.

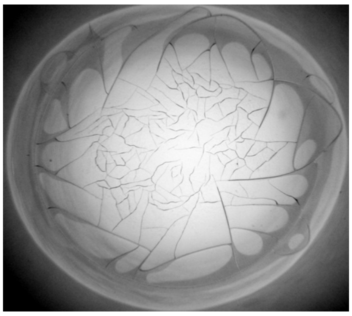

Fig. 3. Facies of serum of patient with cervical cancer after a course of radiotherapy.

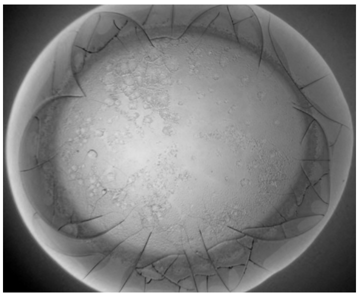

Fig. 4. Facies of serum of patient with cancer of the head and neck at admission to treatment.

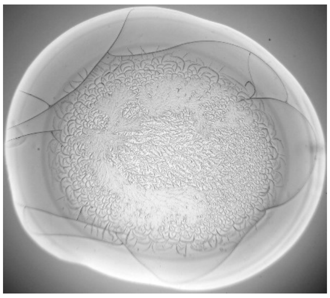

Fig. 5. Facies of serum of patient with cancer of the head and neck in the middle of the course of treatment.

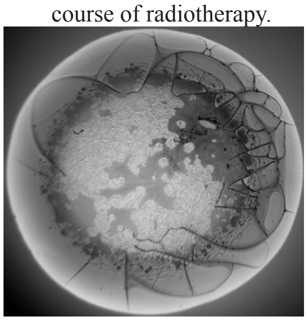

Fig. 6. Facies of serum of patient with cancer of the head and neck after a course of radiotherapy.

Morphological pictures of presented facies of serum of patients with cancer of the head and neck at the stage of admission and discharge are consistent with data of IT.

Conclusions. The total albumin concentration in healthy and cancer patients is within the normal range. Statistically significant change in the values of the effective albumin concentration, the reserve albumin binding and the index of toxicity during radiation therapy reflects the effectiveness of the use of low-cost method of fluorescent probes to quickly obtain information about the dynamics of the flow of the disease process. Wedge dehydration method can be used as an additional way to visualize the effect of external factors on the structural state of biological fluids.

LITERATURE

1. Serum albumin in clinical medicine / Ed. Y. A. Gryzunov, D. E. Dobretsov. M.: IRIUS, 1994. 226 p.
2. *Afanasiyeva A. N., Yevtushenko V. A.* The clinical significance of determining albumin indicators in cancer patients // Anaesthesia and Intensive Care. 2004. N 6. P. 64–67.
3. *Gavrilov V. B., Bidula M. M., Furmanchik D. A.* et al. Evaluation of intoxication of organism by imbalance between accumulation and binding of toxins in the blood plasma // Clin. Lab. Diagnostics. 1999. N 2. P. 13–17.
4. *Gryzunov Y. A., Sachs I. O., Moroz V. V.* et al. Serum albumin: properties, functions, and their evaluation at critical conditions // Anaesthesia and Intensive Care. 2004. N 6. P. 68–74.
5. *Kuzin A. M., Kopylov V. A.* Radiotoxins. M.: Science, 1983. 174 p.

6. *Lukouskaya N. D., Gromyko N. L., Baranovskaya E. I.* et al. Clinical significance of determining the state of binding sites of human serum albumin during pregnancy // News of NAS of Belarus. Series of Med. Sci. 2008. N 3. P. 58–63.
7. *Miller Y. I.* Binding of xenobiotics by serum albumin // Clin. Lab. Diagnostics. 1993. N 1. P. 34–40.
8. *Prokhorova V. I., Tsyrus T. P., Pasko L. I.* et al. Structural and functional state of human serum albumin in the surgical treatment of esophageal cancer // Actual problems of Oncology and Medical Radiology: Collection of Scientific Papers: Minsk, 2000. P. 117–121.
9. Radiotoxins, their nature and role in the biological effects of high energy radiation / Ed. A. M. M. Kuzin. Atomizdat, 1966. 293 p.
10. *Selivanenko V. T., Shatokhina S. N., Dudakov V. A.* Diagnostic value of morphological picture of blood serum in patients with infective endocarditis // Cardiology and Cardiovascular Surgery. 2008. N 2. P. 80–84.
11. *Sidorenko Y. S., Vladimirova L. Y., Frantsiyants E. M.* Effect of chemotherapy on the structure and functional properties of blood serum albumin in patients with breast cancer // Quest. Oncol. 2001. Vol. 47. N 3. P. 303–306.
12. *Sidorov P. I., Kirpitch I. A., Volchetskiy A. L.* Crystallographic studies of blood serum of patients with chronic alcoholism // Addiction. 2002. N 1. P. 9–13.
13. *Smolyakova R. M.* The study of structural and functional state of serum albumin in patients with lung cancer by the method of EPR-spectroscopy // Health. 1998. N 11. P. 17–20.
14. *Smolyakova R. M.* Changes in the physico-chemical characteristics of the conformational state of serum albumin and their clinical significance in patients with lung cancer: Authoref. Dissertation ... PhD: 03.12.01. Minsk, 1999. 21 p.
15. *Tarasevich Y. Y.* Mechanisms and models of the dehydration self-organization in biological fluids // Successes of Physical. Sci. 2004. Vol. 174. N 7. P. 779–790.
16. *Shabalin V. N., Shatokhina S. N.* The morphology of human biological fluids. M.: Chrysostom, 2001. 304 p.

Стаття: надійшла до редакції 14.10.10

доопрацьована 02.03.12

прийнята до друку 05.03.12

КЛІНІЧНЕ ЗНАЧЕННЯ ВИКОРИСТАННЯ МЕТОДІВ ФЛУОРЕСЦЕНТНИХ ЗОНДІВ І КЛИНОПОДІБНОЇ ДЕГІДРАТАЦІЇ В ОЦІНЦІ АЛЬБУМІНОВИХ ПОКАЗНИКІВ ОНКОХВОРИХ

Н. Луковська[1], А. Шафорост[1], А. Маленченко[1], В. Бєляковський[2], Д. Окунцов[2], Т. Пригожа[2], С. Стасенкова[2], Н. Крутіліна[2], Л. Пархоменко[2]

[1]Інститут радіобіології НАН Білорусі
вул. Федюнинського, 4, Гомель РБ 246007, Білорусь
e-mail: irb@mail.gomel.by
[2]Гомельський обласний клінічний онкодиспансер
вул. Медична, 2, Гомель РБ 246012, Білорусь
e-mail: ic@grcoc.gomel.by

Показано, що загальна концентрація альбуміну сироватки крові здорових людей і онкохворих перебуває в межах нормальних значень. Виявлено статистично достовірну зміну показників «ефективна концентрація альбуміну» й «індекс токсичності», яка відображає ефективність проведеної променевої терапії, пов’язану з процесом руйнування пухлинних клітин і надходженням продуктів катаболізму в кров’яне русло. Отримані результати узгоджуються з даними, отриманими методом клиноподібної дегідратації, який може бути використаний для візуалізації стану біологічних рідин і порушення їхніх властивостей при патологічних процесах.

Ключові слова: альбумін, рак шийки матки, рак голови і шиї, ОКА, ЕКА, РСА, ІТ, метод клиноподібної дегідратації.

КЛИНИЧЕСКОЕ ЗНАЧЕНИЕ ИСПОЛЬЗОВАНИЯ МЕТОДОВ ФЛУОРЕСЦЕНТНЫХ ЗОНДОВ И КЛИНОВИДНОЙ ДЕГИДРАТАЦИИ В ОЦЕНКЕ АЛЬБУМИНОВЫХ ПОКАЗАТЕЛЕЙ ОНКОБОЛЬНЫХ

Н. Луковская[1], А. Шафорост[1], А. Маленченко[1], В. Беляковский[2], Д. Окунцов[2], Т. Пригожая[2], С. Стасенкова[2], Н. Крутилина[2], Л. Пархоменко[2]

[1]Институт радиобиологии НАН Беларуси
ул. Федюнинского, 4, Гомель РБ 246007, Беларусь
e-mail: irb@mail.gomel.by
[2]Гомельский областной клинический онкодиспансер
ул. Медицинская, 2, Гомель РБ 246012, Беларусь
e-mail: ic@grcoc.gomel.by

Показано, что общая концентрация альбумина сыворотки крови здоровых людей и онкобольных находится в пределах нормальных значений. Выявлено статистически достоверное изменение показателей «эффективная концентрация альбумина» и «индекс токсичности», которая отражает эффективность проведенной лучевой терапии, связанную с процессом разрушения опухолевых клеток и поступлением продуктов катаболизма в кровяное русло. Полученные результаты согласуются с данными, полученными методом клиновидной дегидратации, который может быть использован для визуализации состояния биологических жидкостей и нарушения их свойств при патологических процессах.

Ключевые слова: альбумин, рак шейки матки, рак головы и шеи, ОКА, ЭКА, РСА, ИТ, метод клиновидной дегидратации.