IRSTI 29.19.00 https://doi.org/10.26577/ijmph.2024v15i2b5

J. Batmyagmar[1], R. Chinzorig[1+], M. Odsuren[1*], O.Tselmeg[1,2], G. Khuukhenkhuu[1], S. Davaa[1], O. Khaliunaa[1], U. Undrakh[3], V. Enkhtsetseg[3], A. Zolbayar[3], Sh. Munkhbat[3], O. Odgerel[1]

[1]School of Engineering and Technology, National University of Mongolia, Ulaanbaatar, Mongolia
[2]Garidmanlai Hospital, Ulaanbaatar, Mongolia
[3]National Cancer Center of Mongolia, Ulaanbaatar, Mongolia
+e-mail: chinzorig@num.edu.mn
*e-mail: odsuren@num.edu.mn

(Received 7 September 2024; accepted 14 October 2024)

Effects of gamma radiation on lung and colon cancer cell viability

Abstract. In Mongolia, cancer treatment is carried out through radiation, chemotherapy and surgery, and there is only one state hospital that specializes in this field, the National Cancer Center of Mongolia. As our country's population has grown in recent years, data for 2023 from the Central Statistical Office show that the incidence of cancer, on the one hand, and the number of cases of the disease, on the other, are increasing as patients get younger. This study concerns an important part of the triad of cancer treatment, namely the evaluation of the efficacy of radiotherapy in an "in vitro" situation. Ionizing radiation has been shown to have a number of biological effects, including damage to DNA. This damage can manifest as double-strand breaks (DSB), base damage and single-strand breaks (SSB). The ^{60}Co radiation source "Bhabhatron", which is installed at the National Cancer Center of Mongolia, is used as a gamma source for the cell viability experiment. In this experiment, we use two different cancer cell samples (A549, HCT116) and all used samples prepared in the Gene Engineering Laboratory of the National University of Mongolia in a quantity of 1 ml, and the cell samples were irradiated with doses of 2 Gy, 4 Gy, 6 Gy and 8 Gy. The cell viability of A549 and HCT116 cancer cells after gamma irradiation was determined using the WST assay and calculated using an analytical formula. The calculated and measured results are comparable for two different cancer cells.

Key words: cancer cells, cell viability, WST assay, cancer treatment, radiation doses.

Introduction

Cancer remains the leading cause of death worldwide and radiotherapy is an important method of cancer treatment [1]. According to the latest GLOBOCAN data from the International Agency for Research on Cancer (IARC), a total of 19.3 million cancer cases were reported worldwide in 2020 and almost 10 million deaths were attributable to cancer. This includes 11.4% lung cancer (the second most common cancer incidence) and 10.0% colorectal cancer (the third most common cancer incidence) [2].

According to the 2022 data released by the Statistics Committee of the National Cancer Center of Mongolia (NCCM), the number of cancer-related deaths has shown a consistent increase over the past two decades in our country. Specifically, lung cancer accounted for 10.6% of newly diagnosed cases, while colon cancer accounted for 3.8%, collectively representing approximately 15% of all cases.

In our country, the treatment of various malignancies is primarily by radiotherapy, chemotherapy and surgery, and this study concerns an important part of the triad of cancer treatment, namely the evaluation of the efficacy of radiotherapy in an "in vitro" situation. Ionizing radiation has been shown to have a number of biological effects, including damage to DNA. This damage can manifest as double-strand breaks (DSB), base damage and single-strand breaks (SSB) [3].

The aim of this study is to investigate the effects of different doses of ionizing radiation on colon (A549) and lung (HCT116) cancer cells. The cells were treated with gamma rays from the

"Bhabhatron", which are frequently used in cancer therapy worldwide. The radiosensitivity of cancer cells A549 (colon) and HCT116 (lung) was determined using the WST assay and the measured results were compared with the calculated values derived using an analytical formula [2-3].

Methods

Experiment

In this work we use A549 and HCT116 cancer cell samples and detailed information of the cells can be found in Ref. [4-7]. All cell samples were prepared in the Gene Engineering Laboratory of National University of Mongolia (NUM) and stored in liquid nitrogen vapor until use. The human lung cancer cell line A549 and the colorectal carcinoma cell line HCT116 were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with a nutrient medium containing 1% penicillin-streptomycin, 10% fetal bovine serum, and 1% L-glutamine. Ten tubes with 1 ml cell sample each were used (the tube with the cell sample had a length of 4.5 cm and a diameter of 1 cm).

The cells were irradiated with gamma rays from the ^{60}Co source "Bhabhatron" (National Cancer Center, Mongolia) the cell samples required 3.8 minutes to 15.3 minutes to absorb doses of 2 Gy to 8 Gy. The general scheme of this experiment is displayed in Fig. 1. Subsequently, the WST assay was used to determine the effect of IR induced cell death [8-10].

The WST assay is based on the cleavage of the tetrazolium salt WST to formazan by cellular mitochondrial dehydrogenases. The larger the number of viable cells, the higher the activity of the mitochondrial dehydrogenases, and in turn the greater the amount of formazan dye formed.

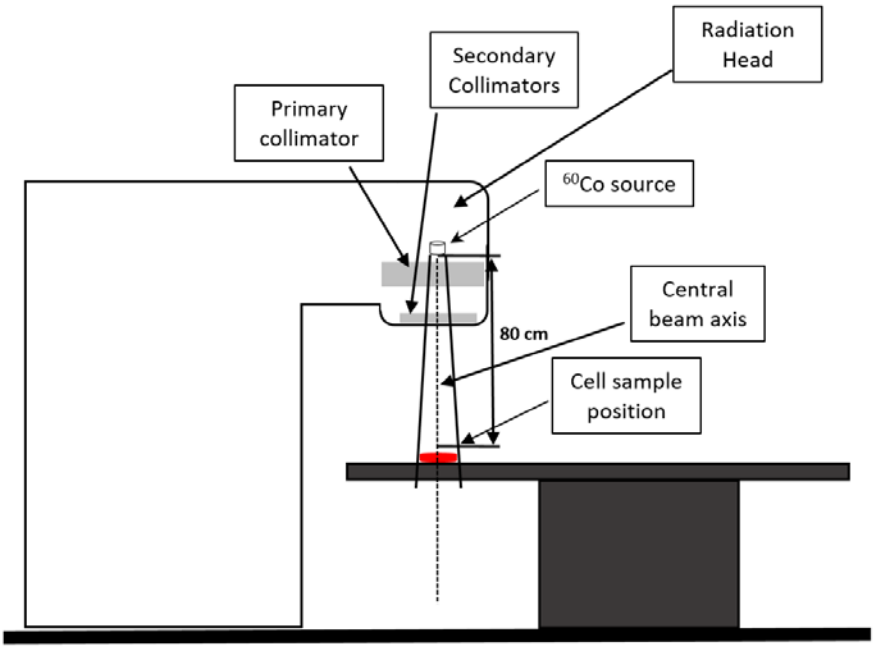

Figure 1 – Scheme of irradiation of cancer cells with gamma rays in Bhabhatron.

Analytical formula

The linear-quadratic (LQ) model is one of the most important tools in radiation biology and physics, as it establishes a relationship between cell survival and the dose delivered [11]. The survival rate of cancer cells depends on the applied radiation dose. Cancer cell viability can be expressed by the following analytical expression, which is a linear-quadratic approximation

$$S = e^{-\eta_{AC} D}, \quad (1)$$

where S – survival rate of cancer cells, η_{AC}- damage leading to immediate cell death (Gy-1), D – radiation dose (Gy).

When a cell is treated to a high dose, the survival equation becomes to [12]

$$-\ln S = (\eta_{AC} + \eta_{AB})D - \\ -\varepsilon \ln\left[1 + \frac{\eta_{AB}D}{\varepsilon}(1 - e^{-\varepsilon_{BA}t_r})\right], \quad (2)$$

and

$$\varepsilon = \frac{\varepsilon_{AB}}{\varepsilon_{BC}}, \quad (3)$$

where: $\eta_{AB}$is damage that can lead to cell death (Gy^{-1}), t_r is the available repair time,ε is the ratio of the constants, and the rate constants for the formation of restored lesions per unit of time. From Eqs. (1) and (2), the cancer cell survival linear quadratic approximation can be written as:

$$-\ln S = (\eta_{AC} + \eta_{AB}e^{-\varepsilon_{BA}t_r})D + \\ + \frac{\eta_{AB}^2}{2\varepsilon}(1 - \varepsilon^{-\varepsilon_{BA}t_r})^2 D^2 \quad (4)$$

where

$$\alpha = \eta_{AC} + \eta_{AB}e^{-\varepsilon_{BA}t_r} \quad (5)$$

and

$$\beta = \frac{\eta_{AB}^2}{2\varepsilon}(1 - e^{-\varepsilon_{BA}t_r})^2, \quad (6)$$

then the absorbed dose rate is

$$\dot{D} = \frac{kSE\frac{\mu_t}{\rho}e^{-\mu D}}{4\pi r^2}. \quad (7)$$

Where: k is collective constant to convert energy fluence rate to dose rate in [Gy/h] and k=5.76·10^7, S is the source strength in [s^{-1}], E is the photon energy in [MeV], μ_t/ρ is the mass absorption coefficient for the material at the dose [cm^2/g], μ is the linear attenuation coefficient for the photons in the shield material [cm^{-1}], D is the thickness of the shield [cm], r is the effective distance of the source from the dose point [cm].

Results and Discussion

Measurement of cell viability

In this work, two different cell samples are used for the cell viability of the experiment, ideally HCT116 and A549 cancer cell samples. All cell (A549, HCT116) samples were irradiated in the Bhabhatron (^{60}Co source), NCCM between 3.18 and 15.3 minutes, depending on the irradiation dose, and the cell samples were prepared as 1 ml in the Gene Engineering Laboratory, NUM. Cancer cell samples irradiated with gamma rays from 2 Gy to 8 Gy were cultured in 96-well cell culture plates for 4 hours, and cell viability was determined for each sample using the WST assay. The microscopic images of A549 and HCT116 cancer cells at 40x magnification on the digital biological microscope are shown in Figure 2, where not only the images of the monitor cell samples (a) A549 and (c) HCT116, but also the cell samples irradiated with 4 Gy (b) A549 and (d) HCT116 are shown.

As shown in Fig. 2 (a) and (c), the shapes of the monitor samples of two different cells are basically circular, but after irradiation with 4 Gy in (b) and (c), the shapes of the two cells are highly deformed, such as ellipsoidal or angled. These changes can be easily seen with a biological digital microscope, and even after irradiation with different doses, the color of the prepared cell samples changes depending on the dose rate; ideally, the color becomes lighter when the cell samples are irradiated with a high dose (Fig. 3). As can be seen in Fig. 3, the color of the prepared samples was strongly pink, as in the N3 monitor sample. The color of these samples then becomes lighter pink and like a liquid with a pink glow when these samples are irradiated with doses of 2 Gy or 6 Gy. This means that the number of cells in the prepared samples decreases depending on the irradiation dose.

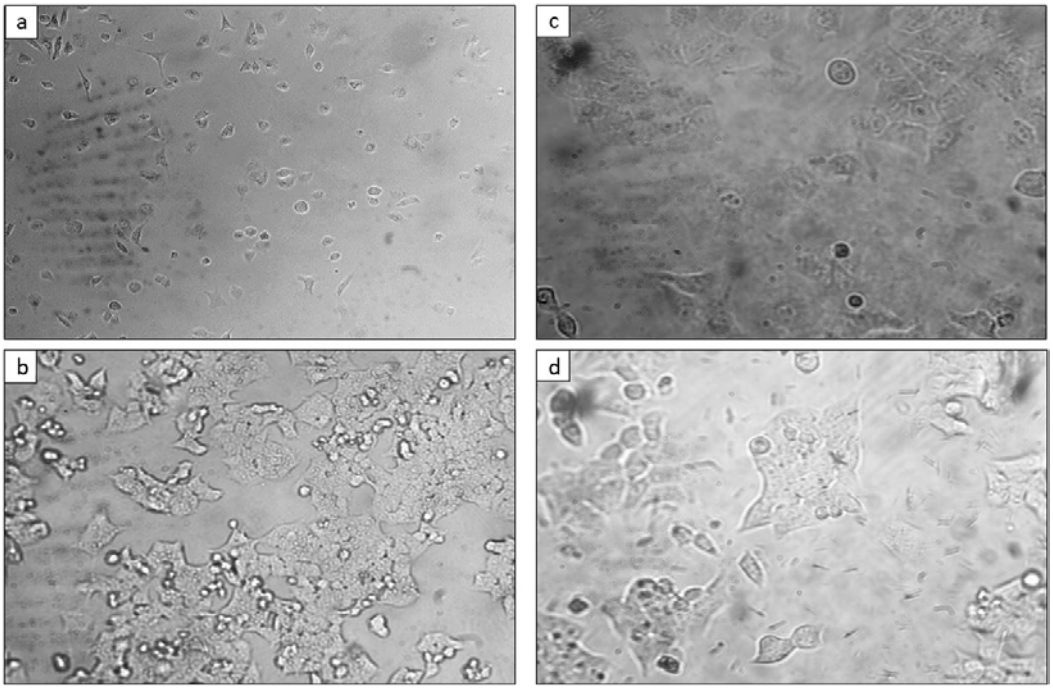

Figure 2 – The A549 and HCT116 cancer cells at 40x magnification on the biological digital microscope: (a) and (c) the monitor cell samples of A549 and HCT116. Cell samples of A549 and HCT116 irradiated with 4Gy are shown in (b) and (d).

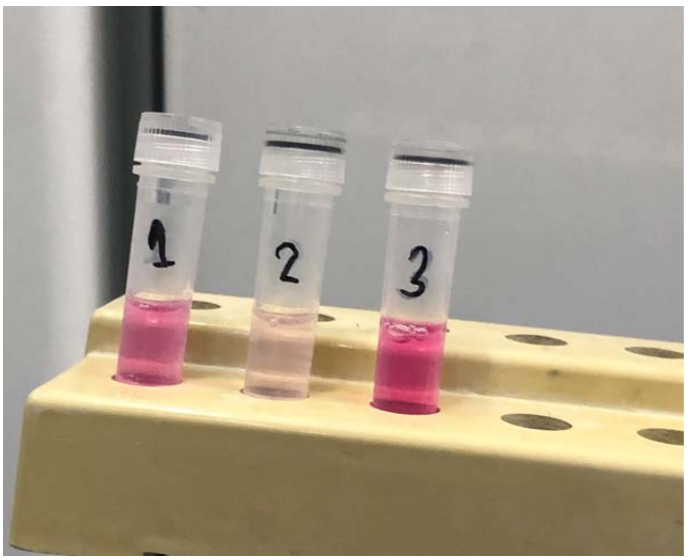

Figure 3 – The cell samples irradiated with different doses and the monitor cell samples (A549). Samples N1, N2 are the samples irradiated with 2 Gy and 6 Gy and the monitor samples (not irradiated).

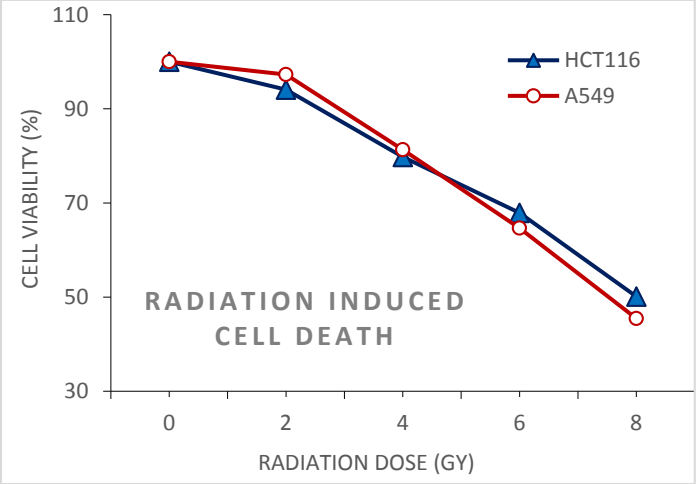

Figure 4 – The WST assay for determining the viability of cells.

In this work, we use the IC_{50}, which requires a radiation dose that reduces the cancer cells in each sample by 50 percent.

The WST assay to determine cell viability is shown in Fig. 4 for different doses between 2 Gy and 8 Gy in steps of 2 Gy for two different cells, and the monitor cell samples were also measured at 0 Gy.

The filled blue triangles and the open red circles show HCT116 and A549 cells. The measurement results show that at low radiation doses (2 Gy, 4 Gy), but this tendency is reversed at higher radiation doses (6 Gy, 8 Gy). As shown in Fig. 4, the cell viability of A549 cells is 97.2 percent at 2 Gy and then gradually decreases to 45.4 percent at 8 Gy. In contrast, the cell viability of NCT116 cells is 94.1 percent at 2 Gy and then decreases to 50.1 percent at a dose of 8 Gy.

Calculation of cell viability

Eq. (7) for photon radiation is used to calculate the gamma radiation dose for the irradiation of 1 ml of a cancer cell sample with 2 Gy photons:

$$\dot{D} = \frac{5.76 \cdot 10^{-7} \cdot 3.2040 \cdot 10^{17} h^{-1} \cdot 2.0027 \cdot 10^{-13} J \cdot 6.32 \cdot 10^{3} \frac{m^2}{kg} \cdot e^{-0.000248 m^{-1} \cdot 0.001 m}}{12.56 \cdot 0.8 m^2} = 28.7 Gy/h$$

where: k=5.76·10^{-7}, S=3.2040 h^{-1}, E=2.0027·10^{-13} J, μ_t/ρ=6.32·10^3 m^2/kg, μ=0.000248m^2, r=0.8 m, D=0.001 m.

The mass absorption coefficient and linear attenuation coefficient were taken from NIST, and 0.0478 Gy/min is used for the Bhabhatron dose rate.

The irradiation time for 2 Gy as a radiation dose in the cells is determined as follows:

$$t = \frac{D}{\dot{D}} \rightarrow \frac{2Gy}{0.478 Gy/minmin.}$$

The viability of 1 ml A549 and HCT116 cancer cell samples can be calculated using Eq.(4), where the A549 cell is irradiated with a dose of 2 Gy:

$$-\ln S_2 = 0.1242 Gy^{-1} \times 2Gy - -0.0234 Gy^{-2} \times 2Gy^2,$$

the above expression is simplified as

In this study, the viability of cancer cells irradiated with different doses of radiation were calculated by selecting appropriate values for the cell survival parameters α and β in Eq (4), which depend on the nature of the cancer cells.

The cell survival parameters used in this work are listed in Table 1, and Table 2 summarizes not only the calculated results for the cell viability of A549 and HCT116, but also the measured results.

The cell survival parameters for the A549 and HCT116 cells are listed in Table 1 and are used for the calculation of cell viability.

Table 1 – Cell survival parameters for the A549 and HCT116 cell

Cell samples	Constant values used for the calculation			
	α	β	α/β	SF
A549	0.056	0.048	1.2	0.816
HCT116	0.20	0.036	0.5	0.823

Table 2 – Calculated and measured cell viability of A549 and HCT116 cells

A549 cell				
Cell samples (Irradiated dose)	Radiation dose [Gy]	Irradiation time [min]	Cell viability [%]	
			Calculated	Measured
1st sample (2 Gy)	2.0	3.8	81.6	97.2
2nd sample (4 Gy)	4.0	7.6	59.3	81.2
3rd sample (6 Gy)	6.0	11.5	45.7	64.6
4th sample (8 Gy)	8.0	15.3	23.5	45.4
Monitor cell sample	0.0	0.0	100.0	100.0
HCT116 cell				
1st sample (2 Gy)	2.0	3.8	82.3	94.1
2nd sample (4 Gy)	4.0	7.6	72.1	79.7
3rd sample (6 Gy)	6.0	11.5	68.5	67.8
4th sample (8 Gy)	8.0	15.3	36.9	50.1
Monitor cell sample	0.0	0.0	100.0	100.0

The results of the calculated and measured cell viability are shown in Table 2, and the results of the A549 and HCT116 cells are compared at various doses between 2 Gy and 8 Gy in steps of 2 Gy. Samples of monitor cells are kept under the same conditions during irradiation but are not irradiated.

As can be seen from the measurements of A549 and HCT116 cells, the measured cell viability of both cells is slightly higher than the calculated cell viability at all different doses, but consistent with each other.

The calculated HCT116 cell viability is always high compared to the A549 cell viability at all doses except the highest dose (8 Gy). This tendency is reversed for the measured cell viabilities in the ideal case, i.e. the A549 cell viability is higher than the HCT116 cell viability at the lowest doses between 2 and 4 Gy, but at the high doses between 6 and 8 Gy it is low compared to the HCT116 cell viability.

Summary

The A549 and HCT116 cancer cell samples (1 ml) are prepared in the Gene Engineering Laboratory, NUM, and these prepared cancer cell samples are irradiated between 3.18 and 15.3 minutes, depending on the irradiation dose of 1 Gy to 8 Gy generated by a gamma radiation source from the Bhabhatron (60Co source), NCCM.

The viability of A549 and HCT116 cancer cells was determined not only by WST assay but also by biological digital microscope, where the shape of cells before gamma ray irradiation is essentially circular, while after irradiation it is ellipsoidal or angled, which can be easily seen by biological digital microscope.

We found that the measured cell viability for the A549 and HCT116 cells are consistent with each other. It might be helpful if we use other cancer cell samples such as liver, breast and kidney cancer cells in future work.

Acknowledgement

This work has been done within the framework of the project (P2024-4678) supported by the Asia Research Center at the National University of Mongolia and Chey Institute for Advanced Studies, Korea.

References

1. Baskar Rajamanickam, Kuo Ann Lee, Richard Yeo, and Kheng-Wei Yeoh. "Cancer and Radiation Therapy: Current Advances and Future Directions." *International Journal of Medical Sciences* 9 (2012): 193–199.

2. Sung Hyuna, Jacques Ferlay, Rebecca L., Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal, and Freddie Bray. "Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries." *CA: A Cancer Journal for Clinicians* 71 (2021): 209–249.

3. Teoule, R. "Radiation-Induced DNA Damage and Its Repair." *International Journal of Radiation Biology and Related Studies in Physics, Chemistry and Medicine* 51 (2009): 573–589.

4. Phillips Joanne R., and Kevin M. Prise. "DNA Damage Response in Human Cells Exposed to Ionizing Radiation." *Nature Reviews Cancer* 4, no. 11 (2004): 861–872.

5. O'Neill Peter, and E. Martin Fielden. "Primary Free Radical Processes in DNA." In *Advances in Radiation Biology*, 17 (1993): 53–120.

6. Selenius Lisa Arodin, Marita Wallenberg Lundgren, Rim Jawad, Olof Danielsson, and Mikael Björnstedt. "The Cell Culture Medium Affects Growth, Phenotype Expression and the Response to Selenium Cytotoxicity in A549 and HepG2 Cells." *Antioxidants* 8 (2019): 130.

7. Manda Katrin, Tina Präkelt, Tonja Schröder, Stephan Kriesen, and Guido Hildebrand. "Radiosensitizing Effects of Trabectedin on Human A549 Lung Cancer Cells and HT-29 Colon Cancer Cells." *Investigational New Drugs* 38 (2020): 947–976.

8. Präbst Konstantin, Hannes Engelhardt, Stefan Ringgeler, and Holger Hübner. "Basic Colorimetric Proliferation Assays: MTT, WST, and Resazurin." In *Methods and Protocols, Methods in Molecular Biology*, 1601 (2017): 1–17.

9. J. Allison, K. Amako, J. Apostolakis, et al. "Recent Developments in Geant4." *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment* 835 (2016): 186–225.

10.*NIST Chemistry Web Book*. NIST Standard Reference Database Number 69. https://doi.org/10.18434/T4D303.

11.McMahon Stephen Joseph. "The Linear Quadratic Model: Usage, Interpretation and Challenges." *Physics in Medicine and Biology* 64 (2019): 1–24.

12.Alpen Edward L. *Radiation Biophysics*. 2nd ed. California: Department of Biophysics, Emeritus University of California, 1990. pp. 164–165.

Information about authors:

Batmyagmar J. – Master student, National University of Mongolia, e-mail: batmyagmar.j@num.edu.mn

Chinzorig R. – Doctor of Biology, Associate Professor, National University of Mongolia, e-mail: chinzorig@num.edu.mn

Odsuren M. – Doctor of Nuclear Physics, Associate Professor, National University of Mongolia, e-mail: odsuren@num.edu.mn

Tselmeg O. – Master student, National University of Mongolia, e-mail: hashhan@gmail.com

Khuukhenkhuu G. — Doctor of Physical and Mathematical Sciences, Professor, National University of Mongolia, e-mail: khuukhenkhuu@num.edu.mn

Davaa S. – Doctor of Physical and Mathematical Sciences, Professor, National University of Mongolia, e-mail: davaa@num.edu.mn

Khaliunaa O. – Master student, National University of Mongolia, e-mail: haliunaa@gmail.com

Undrakh U. – Master student, National University of Mongolia, e-mail: undrakh_1202@yahoo.com

Enkhtsetseg V. – Medical Physicists, National Cancer Center, Mongolia, e-mail: v.enkhtsetseg@gmail.com

Zolbayar A. – Medical Physicists, National Cancer Center, Mongolia, e-mail: zolbayar.a@gmail.com

Munkhbat Sh. – Medical Physicists, National Cancer Center, Mongolia, e-mail: munkhbat.sh@gmail.com

Odgerel O. – Doctor of Biology, Associate Professor, National University of Mongolia, e-mail: odgerelo@num.edu.mn