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## **Redox-dependent Markers for Response to Neoadjuvant Radiotherapy for Rectal Cancer**

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### **ABSTRACT**

Biopsy materials of tumors and urine samples from 26 patients with rectal adenocarcinoma of the stage T<sub>2-4</sub>N<sub>0</sub>M<sub>0</sub> have been examined. In the first phase of treatment, patients received a preoperative course of radiotherapy to the tumor to a total focal dose of 39 Gy (single focal dose 3 Gy with 13 sessions during 2.5 weeks). A radical surgical intervention was done after 4-5 weeks of treatment. The results of this research single out new prognostic criteria of the tumor radiosensitivity level. Superoxide radicals speed production value before the treatment more than 1,0 nmol/min·body weight in g. of raw tissue; the low level of the 8-oxoG daily excretion in the urine before the treatment – less than 0,5 nmol/period·body weight in kg; and the increasing of the 8-oxoG index level in the urine at 50% and more relative to the baseline the day after the beginning of radiotherapy points at the high level of tumor radiosensitivity.

**Keywords:** Rectal cancer, Neoadjuvant Radiotherapy, Radiosensitivity, Predictive assay, Superoxide anion, 8-oxo-guanine.

### **1. Introduction**

In the course of the last 15 years the colorectal cancer firmly holds the second-third place in the most economically developed countries as of its place in the cancer disease and mortality structure. It accounts for 9.4% of all malignancies in men and 10.1% in women. There have been registered 1.4 million new cases of it in the year 2012 of which 23-38% falls on rectal cancer. According to epidemiological studies the number of colorectal cancer cases among the population has been steadily increasing over the past 50 years in the majority of countries, mainly in those who are economically successful ones, except for the USA [1, 2, 3]. It is anticipated that by the year 2035 there will be diagnosed 2.4 million new cases of colorectal cancer annually all over the world [4].

Surgery remains the leading treatment method of the colorectal cancer. But the unsatisfactory long-term outcomes and high recurrence rate urge to use different combined methods. The key component of the combined treatment of rectal cancer is a preoperative radiotherapy, the efficiency of which is largely dependent on tumor radiosensitivity. However, despite the long history of the research, the selection of appropriate and highly informative biomarkers, the use of which would provide prospective prediction of tumor response to radiation therapy, is a problematic one. The administration of the radiation treatment in different total focal doses and fractionation regimen is based on routine clinical characteristics mainly. They include the site and the dimensions of primary tumor, the histological type and tumor's differentiation degree, the general condition of the patient, and the stage of the oncological disease [5]. The prediction of the radiation sensitivity level at the early stages of radiotherapy is one of the most promising directions in improving the treatment of rectal cancer, since the affirmation of tumor radioresistance should prevent the loss of time for irradiation due to a delay of operation or vice versa, to justify the need for increasing doses of radiation or the use of additional radio modifying agents [6].

Diagnostic and prognostic significance of direct monitoring of the superoxide anion radicals (SAR) in a tumor tissue and the determination of the oxidative DNA modifications products level, including 8-oxoguanine (8-oxoG) at the system level, is not sufficiently studied yet. 8-oxoG is a sensitive biomarker of oxidative DNA damage in the body and its registration in the urine allows us to estimate the intensity of the genotoxic intracellular processes [7, 8, 9, 10]. Though at present the number of clinical researches relating to the study of this marker is relatively small, they showed an increase excretion of 8-oxoG in the urine of cancer patients undergoing a radiotherapy [11, 12, 13].

## 2. Materials and Methods

Biopsy materials of tumors and urine samples from 26 patients (11 men and 15 women, mean age  $62 \pm 1,8$  years) have been examined. They were diagnosed with rectal adenocarcinoma of the II stage ( $T_{2-4}N_0M_0$ ), when undergoing the treatment at Ivano-Frankivsk Regional Clinical Oncology Center (Ukraine). In the first phase of treatment, patients received a preoperative course of gamma irradiation to the tumor region to a total focal dose of 39 Gy. A single focal dose constituted 3 Gy with 13 sessions during 2.5 weeks, 5 sessions per week. A radical surgical intervention was done after 4-5 weeks of treatment. The exclusion criteria of the patients under the examination were the following: those who were over 80 years old; multiple primary character of tumor lesions; common grave condition of the patients due to the presence of accompanying systemic diseases in the stage of decompensation; intake of antioxidant vitamins by them a month before and during the research. It was conducted in compliance with the principles of biomedical research involving human subjects as indicated in the Declaration of Helsinki (1964) of the World Medical Association.

The SAR speed production in homogenate of bioptic material was recorded by electron paramagnetic resonance at room temperature applying the Spin Traps technology and spin trap 1-hydroxy-2,2,6,6-tetramethyl-4-oxopiperidine (Russia) [14,15].

The quantitative level of oxidative DNA damage in patients was researched by identifying the 8-oxoG-marker in the daily urine prior to the initiation of the radiotherapy and the day after the patients have received the first fraction of radiation. In order to do this, 20 ml of filtered daily urine was run through the column for solid-phase extraction of 8-oxoG, followed by a spectrophotometry with the registration of absorption at 250, 260, 280 and 293 nm. The level of 8-oxoG formation in the cells was determined by the formula:

$$R_{ex} = \frac{V \cdot \sum(C_i \cdot V_i)}{V_{appl} \cdot W} \quad (\text{nmol/period} \cdot \text{body weight in kg}),$$

Where  $V$  stands for the total volume of urine in ml.;  $V_i$  – for the urine volume from which the oxidative guanine is separated, ml;  $V_{appl}$  – the urine volume analyzed at the column, ml;  $W$  – the body weight, g;  $C_i$  – the 8-oxoG concentration [16].

The effectiveness of neoadjuvant course of radiotherapy was estimated one month after the end of it, considering the level of therapeutic pathomorphosis in tissue specimen of surgical material while applying the Lavnikova's technique [17].

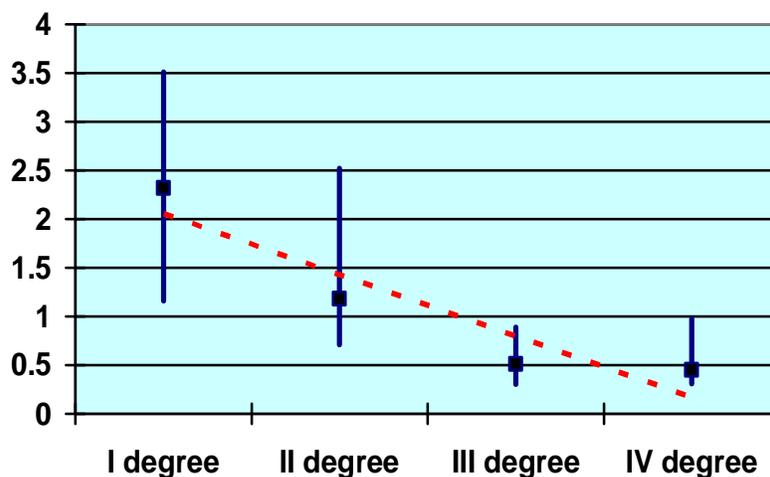
## 3. Results and Discussion

It has been discovered that the day after the beginning of radiotherapy the excretion of 8-oxoG in the urine of rectal cancer patients has considerably increased. Upon dividing the patients into groups depending on the therapeutic pathomorphosis options, it has been revealed that the 8-oxoG index evolution matched the clinical effectiveness of gamma-ray therapy. This index was at its highest in the III-IV stage pathomorphosis cases (1.9-2.8 times growth), and significantly low in cases of the I-II stage (1.2-1.5 times increase) (Table 1).

One should take notice that the absolute figures of the 8-oxoG urine excretion in patients with high efficiency of radiotherapy were relatively low at the early stages of treatment. This was caused by low baseline excretion of the oxidative DNA modifications products. For example, while at the time of hospitalization the 8-oxoG rate in patients with the I stage of pathomorphosis was  $2,32 \pm 0,25$  nmol/period·body weight in kg, it was much less in patients with the IV stage, averaging  $0,45 \pm 0,03$  nmol/period·body weight in kg (Fig. 1).

**Table 1:** The SAR and 8-oxoG indexes level in rectal cancer patients before the treatment and the day after the 1<sup>st</sup> session of the radiation intervention.

The degree of pathomorphosis	Index	Index level before the radiotherapy	Index level after the radiotherapy	P
I (n=6)	SAR (nmol/min·raw tissue in g.)	0,71±0,04 (0,50-1,25)	-	
	8-oxoG (nmol/period·body weight in kg)	2,32±0,25 (1,16-3,51)	2,77±0,17 (1,23-4,75)	>0.05
II (n=7)	SAR (nmol/min·raw tissue in g.)	0,96±0,07 (0,58-1,64)	-	
	8-oxoG (nmol/period·body weight in kg)	1,38±0,11 (0,71-2,52)	2,21±0,23 (1,44-4,32)	<0.05
III (n=7)	SAR (nmol/min·raw tissue in g.)	1,85±0,13 (1,15-2,44)	-	
	8-oxoG (nmol/period·body weight in kg)	0,51±0,04 (0,30-0,89)	0,98±0,06 (0,55-1,42)	<0.01
IV (n=6)	SAR (nmol/min·raw tissue in g.)	3,19±0,26 (2,35-4,17)	-	
	8-oxoG (nmol/period·body weight in kg)	0,45±0,03 (0,31-0,98)	1,26±0,11 (0,65-1,93)	<0.01

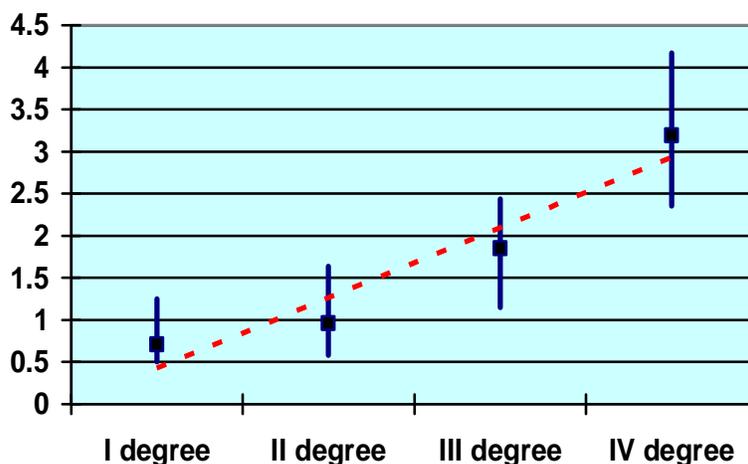


**Fig 1:** The 8-oxoG excretion level of rectal cancer patients before the treatment in accordance with the degree of pathomorphosis (the mean value, the minimum – the maximum, the trendline).

As is generally known, the DNA fragments containing 8-oxoG can be actively excreted by the viable cells due to the enzymatic restoration of DNA damage including by way of nucleoside and excision of DNA by glycosylases. Thus, the low levels of the 8-oxoG daily excretion in the urine before the treatment reflect the low efficiency of DNA repair protection systems, and are the basis for the high radiosensitivity of tissues. If we take into account that the basis of the therapeutic effect of radiation treatment is the biological macromolecules oxidative damage done by the water radiolysis products, it is quite logical that the dynamics in markers' level increase of oxidative DNA damage, the intensity of which is significantly increasing as a result of exposure to radiation, may correlate with the level of therapeutic effect. The main source of 8-oxoG in the extracellular space are the cells, that release the oxidative DNA while being destroyed by apoptosis or necrosis and replenishing its extracellular pool. A significant relative increase of the 8-oxoG excretion the day after the start of

treatment affirms a high level of tissue radiolesion in the radiation zone against the massive free radical damage to the genetic apparatus of the cells.

The examination of tumor biopsies obtained from rectal cancer patients prior to the treatment detected that the higher the initial level of SAR production in neoplasm's tissue is the more chances one has to achieve a high degree of therapeutic pathomorphosis owing to radiotherapy. The average value of the SAR in patients with the therapeutic pathomorphosis of the I degree was  $0,71 \pm 0,04$  nmol/min·g raw tissue before treatment, and the maximum in the cases of the IV stage –  $3,19 \pm 0,26$  nmol / min·g raw tissue (Fig. 2). This indicates that during carcinogenesis the increased tissue production of SAR promotes oxidative phenotype, required for the aggravation of the tumor focus [16], the SAR level appears to be one of the key factors through which cytolytic and cytostatic effects of radiotherapy intervene at the stage of treatment.



**Fig 2:** The SAR level in the tumor tissue of the rectal cancer patients before the treatment in accordance with the degree of pathomorphosis (the mean value, the minimum – the maximum, the trendline).

The results of this research may single out new prognostic criteria. Their comprehensive analysis may indicate the level of tumor radiosensitivity. Superoxide radicals speed production value before the treatment more than 1,0 nmol/min·body weight in g. of raw tissue; the low level of the 8-oxoG daily excretion in the urine before the treatment – less than 0,5 nmol/period-body weight in kg; and the increasing of the 8-oxoG index level in the urine at 50% and more relative to the baseline the day after the beginning of radiotherapy points at the high level of tumor radiosensitivity.

#### 4. Conclusions

So, the data obtained in the course of the research allowed undertaking a comprehensive assessment of the superoxide radicals' production, the intensity of their damaging effects on the cells genetic apparatus in synergy with ionizing radiation, and determining the efficiency of the DNA repair in rectal cancer patients. The valuation of the SAR activity generation systems in tumor tissue and the DNA oxidative damage level in cancer patients can be used as the integral indicator, which enables the prospective prediction of the neoplasm's sensitivity towards the cytostatic impact. This will ensure the timely implementation of the appropriate improvement to the treatment and achieving a maximum degree of therapeutic neoplasm's pathomorphosis.

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