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PRELIMINARY STUDY ON BIOMARKERS OF OXIDATIVE STATUS IN PATIENTS WITH COLORECTAL CANCER

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ABSTRACT

Colorectal cancer is a major cause of mortality and morbidity all over the world and is the third most common cancer and second leading cause of cancer death. Oxidative damage to DNA, proteins, cell membranes and mitochondria are involved in carcinogenesis, but there is insufficient comparative data for the levels of biomarkers of oxidative stress in the tumor and unchanged colon regions tissues in patients with colorectal cancer. By this research we report our preliminary study on *ex vivo* determined levels of catalase (CAT) and superoxide dismutase (SOD) enzyme activities, the level of lipid peroxidation products measured as malone dialdahyde (MDA) reactive products and levels of protein carbonyl content *(*PCC) in cancer and unchanged colon regions tissues isolated from colorectal cancer patients. 12 patients with colorectal cancer have been enrolled in the current study. Statistical significant decreased levels of CAT and SOD enzyme activities were found in tumor tissues comparing to the control tissues of the patients, while no statistical significant difference was found for the levels of MDA. Increased levels of PCC were registered in the tumor tissues of the patients in comparison with those of the control tissues. Based on the results obtained we consider that further detailed studies on the levels of other oxidative stress biomarkers such as: different ROS products, ratio ascorbic acid/ascorbate radicals, and est. would provide additional information on the oxidative status in colorectal cancer patients and would help in the search of a more effective antioxidant therapy to those patients.

Key words: lipid peroxidation products; catalase; superoxide dismutase; protein carbonyl content; colorectal cancer.

INTRODUCTION

Colorectal cancer is one of the most frequent neoplastic diseases in human population and one of the frequent causes of death. Although recent advances achieved in early diagnosis and treatment, rates of mortality have changed little (1). There are a lot of pathological factors, including reactive oxygen species (ROS) involved in the process of cancer initiation and progression. ROS, such as $O_2^{\bullet-}$, H_2O_2, $OH\cdot$, have highly harmful effects on cellular macromolecules like DNA, proteins and lipid (2). Adverse effects and biological damage caused by these species is called oxidative stress (3). These effects of ROS are balanced by the antioxidant enzymes and supported by antioxidant action of non-enzymatic antioxidants. Despite antioxidant defense system to counteract oxidative damage from ROS, oxidative damage accumulates during the life cycle and radical related damage to DNA, proteins and lipids has been proposed to play a major role in the progress of disease such as cancer, atherosclerosis, neurodegenerative diseases and other conditions (4). On the other hand low levels of ROS are irreplaceable as mediators in many cell processes, including differentiation, cell cycle progression or growth arrest, apoptosis and immunity (5).

In the last years, studies have shown that free radical mechanisms could be responsible for initiation and development of certain cancers, including colorectal cancer (6). These

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suggestions are proposed because ROS can damage all cell structures, especially injurious consequences of this type of interaction observed in the event of proteins because they perform a number of regulatory functions in many biochemical processes (7). Under the effect of ROS occurs to damage the structure of the protein chain, oxidative changes of amino acid residues and prosthetic groups. This can lead to rupture polypeptide chain, making cross-linkages in the same chain or other polypeptide chains and structural changes in the amino acids and components of complex proteins, the result of which they can lose their functions (7). The oxidative damage of proteins involves oxidative scission, loss of histidine residues, the introduction of carbonyl groups, and the formation of protein-centered alkyl R•, alkoxyl RO•, and alkylperoxyl ROO•, radicals (8). Oxidatively damaged proteins are not removed by proteosomic systems in an efficient manner and their accumulation in the cell may impair its proper operation. Thus accumulation of oxidized protein products can play an important role in the pathogenesis of cancer (7).

Other targets of ROS are the polyunsaturated fatty acids in the membrane lipids. ROS can react with fatty acids and form lipid peroxides. The decomposition of peroxidized lipids yields leads to wide variety of end-products, including MDA which are widely used in practice as an indicator of free radical damage in membrane polyunsaturated fat acids (PUFA) (9). MDA can react with DNA bases dG, dA, and dC to form adducts M_1G, M_1A and M_1C respectively (8), such reactions may lead to cytotoxicity, mutagenicity and/or carcinogenicity (10).

Insufficient comparative data on the levels of oxidative stress biomarkers in tumor and unchanged tissues regions of cancer patients led us to carry out the present preliminary study to evaluate and compare the levels of PCC, MDA reactive products and enzyme activities of CAT and SOD in cancer and unchanged colon regions tissues isolated from colorectal cancer patients.

MATERIALS AND METHODS

PATIENTS AND BIOLOGICAL MATERIAL

Studies were conducted in primary colorectal cancers tissues samples from 12 patients with colorectal cancer (6 males and 6 females aged 61 ± 5 years) in II clinical stage. As a control, the same amount of samples was collected from macroscopically unchanged colon area of the most distant location to the cancer. Conventional histopathological parameters, including TNM stage, tumor type and grade of differentiation, were assessed by pathologist. Differentiation and histological type of the cancer were determined following the World Health Organization guidelines.

Tissues samples were homogenized and after centrifugation supernatants were studied for some of oxidative stress biomarkers.

Informed consent was obtained from all patients in the study according to the ethical guidelines of the Helsinki Declaration.

LABORATORY ANALYSIS

Estimation of the lipid peroxidation products

The total amount of MDA reactive products in tissues samples of patients was determined by the tiobarbituric acid (TBA) method according to Plaser and Cushman 1966, with some modification by Gadjeva et al., (11). Results were expressed as micromols per mg protein MDA (μM/mgPr).

Determination of Superoxide dismutase activity

SOD activity in the tissue homogenate was measured as described by Sun et al. with minor modifications (12). The xanthine/xanthine oxidase system was used to generate the superoxide anion. This anion reduces nitroblue tetrazolium (NBT) to formazan, which is detected at 560 nm. SOD of the sample removes the superoxide anion and inhibits the reduction. The level of this reduction was used to measure SOD activity (13). The final concentrations of xanthine, xanthine oxidase, and nitroblue tetrazolium in the assay were 50 μM, 10 U/ml, and 0.125 mM. One unit of enzymatic activity was defined as an amount of that causes 50% inhibition of NBT reduction to formazin. The results were expressed as international units per mg of total protein (IU/mgPr).

Catalase activity determination

CAT activity in the tissue homogenate was estimated by the method described by Beers and Sizer (14). Briefly, hydrogen peroxide (30 mM) was used as a substrate and the decrease in its concentration at 22°C in phosphate buffer (50 mM, pH 7.0) was followed

spectrophotometrically at 240 nm. One unit of CAT activity was defined as enzyme amount that degraded 1 μM H_2O_2 per minute. Results were expressed as international units per mg of total protein (IU/mgPr).

Estimation of protein carbonyl content

Determination of PCC was performed by a competitive ELISA kit (OxiSelect™ Protein Carbonyl ELISA Kit: *Cell Biolabs, INC*.). The quantity of protein carbonyl content in samples is determined by comparing its absorbance with that of a known reduced/oxidized BSA standard curve. Each sample was tested in duplicate, and the carbonyl content expressed in nmol/mg protein is the arithmetic mean of two measurements

Statistical analysis

Statistical analysis was performed with Statistica 7, StaSoft, Inc. And the results were expressed as means ± S.E. Statistical analysis was performed with Student's t-test. $P < 0.05$ was considered statistically significant.

RESULTS

Statistical significant decreased levels of CAT (mean 8.66 ± 1.33, vs, mean 16.4 ± 2.59, p=0,004, **Figure 1**) and SOD (mean 4.28 ± 1.71, vs, mean 10.72 ± 1.28, p=0.044, **Figure 2**) enzyme activities were found in tumor tissues comparing to the control tissues of the patients. Quite contrary, increased levels of PCC were registered in the tumor tissue of the patients in comparison with those of the control tissues (mean 5, 51 ± 0.48, vs, mean 5, 19 ± 0.19) **Figure 3**. Moreover as is seen on **Figure 4** decreased levels of MDA products were registered in the tumor tissues comparing to the control tissues of the patients (mean 1,89 ± 0.13, vs, mean 2,04 ± 0.22).

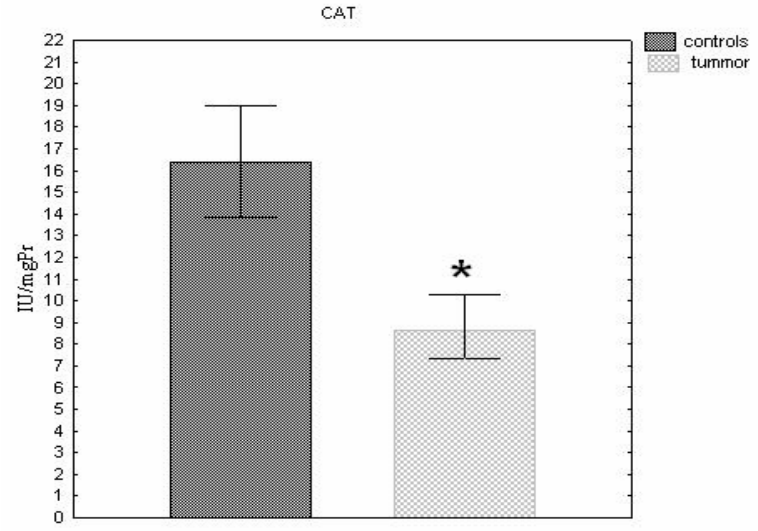

Figure 1. Levels of CAT activity in tumor and control tissues of colorectal cancer patients

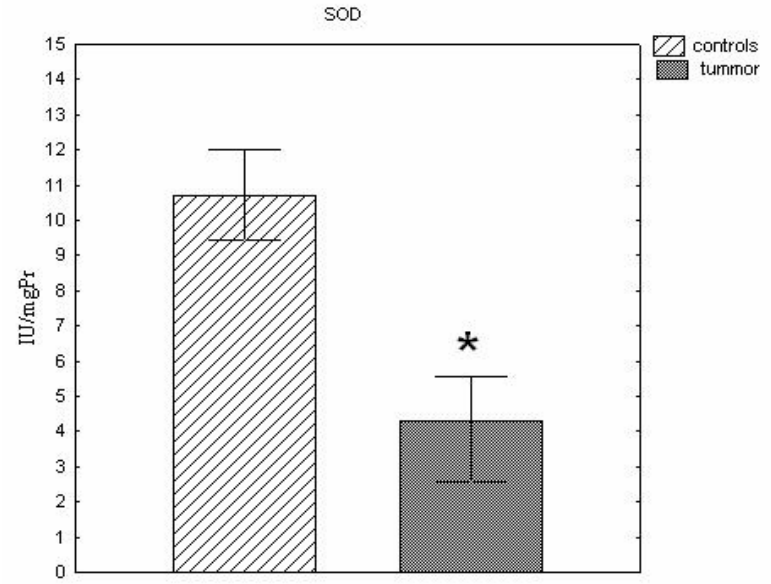

Figure 2. Levels of SOD activity in tumor and control tissues of patients with colorectal cancer.

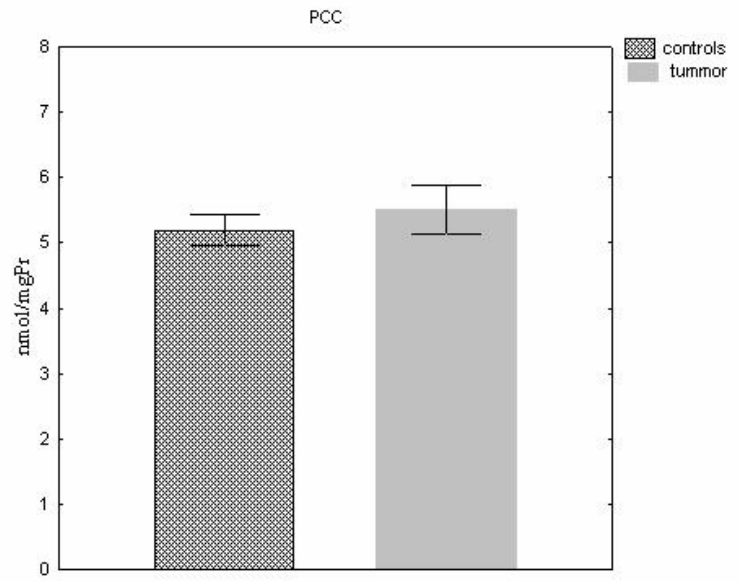

Figure 3. Levels of protein carbonyl content *(*PCC) in cancer and unchanged colon regions of tissues isolated from colorectal cancer patients.

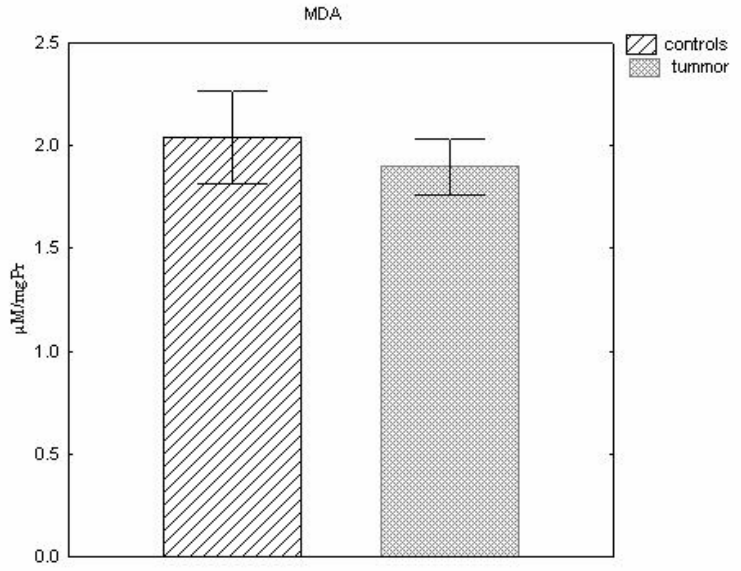

Figure 4. Levels of MDA in cancer and control tissues of colorectal cancer.

DISCUSSION

A great number of studies have been conducted to evaluate the oxidative stress biomarkers in patients with colorectal cancer, but the measurements were performed either in plasma or serum, and the results were compared with those of healthy volunteers. One of the first studies to assess the content of carbonyl groups in serum proteins of cancer patients was carried out in 1998 by a team of Polish researchers – Popadiuk et al. They found two times higher content of carbonyl groups in serum proteins of patients with cancer compared with healthy controls (15). Similar results have been reported recently by Murlikiewicz et al. Authors studied the content of carbonyl groups in serum proteins in patients with cancer of the rectum, and established statistical significant higher levels of PCC comparing to those of healthy subjects (7). Gönenç et al. studied the biomarkers of oxidative stress in 59 patients with newly diagnosed gastrointestinal cancer and compared them to a group of 20 healthy volunteers. In patients with cancer found a statistically higher levels of 8-hydroxy-deoxyguanosine and statistically lower levels of superoxide dismutase and catalase activities compared to the controls (16). Actually, these studies on oxidative stress biomarkers determined in plasma and serum describe the oxidative status of the whole organism, while our research describes the oxidative status of regions whose cells have lost their differentiation compared with the unchanged region of the same patient. Although the

present study was done with tissue samples the results obtained meet the above mentioned research carried out in blood samples. Our data showed statistical significant decreased levels of CAT and SOD enzyme activities in tumor tissues comparing to the control tissues of the patients. Increased levels of PCC found in the tumor tissue samples **(Fig. 3)** might be partially related with the low enzyme activity of SOD and CAT (proteins) due to oxidative formation of carbonyl groups in the enzymes structures. The lower level for MDA products measured in tumor tissues of the patients comparing to the unchanged tissue regions **(Fig. 4)** we might explain by the well established fact that MDA substances are unstable end products of the lipid per oxidation processes (17). We suppose that in tumor tissues because the presence of a variety of oxidative stress end products derived from different bio macromolecules (DNA, proteins, and enzymes) might be a reason for a fast destroying of the MDA products confirmed by our study. This supposition is also indirectly supported by the higher levels of PCC registered in tumor tissues comparing to the unchanged regions **(Fig.3)**.

As the changes of the studied oxidative stress biomarkers in tumor tissues in comparison with those measured in the unchanged tissues of colorectal patients (reported by our present study) are in the same directions as the changes of the same biomarkers measured in the blood samples of colorectal patients comparing to those of healthy volunteers (others authors' study) we assume a similarity in the mechanisms of the oxidative processes in both studies.

CONCLUSIONS

Based on these preliminary results we consider further complex evaluations on the levels of other oxidative stress biomarkers such as: ratio of ascorbate radicals/ascorbic acid, different ROS products ($O_2^{\bullet -}$, H_2O_2, OH^-) and est., would provide additional and more precise information on the oxidative status in colorectal cancer patients and would help in the search of more effective antioxidant therapy to those patients.

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