**Original article**

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# PARAMETERS OF OXIDATIVE STRESS IN COLON CANCER TISSUE

*Andrej Veljković[1], Goran Stanojević[1,2], Branko Branković[1,2], Dušica Pavlović[1], Ivana Stojanović[1], Tatjana Cvetković[1,2], Tatjana Jevtović[1], Dušan Sokolović[1], Jelena Bašić[1], Milena Despotović[1], Branka Đorđević[1], Gordana Kocić[1]*

Colorectal cancer is one of the most frequent neoplastic diseases in the human population, and one of the most frequent causes of death. Reactive oxygen species (ROS) are involved in the process of cancer initiation and progression. It is known that ROS are formed in excess in chronic diseases of the gastrointestinal tract, but the precise mechanisms of oxidative stress being induced in cancer cells and the role of ROS in colorectal cancer progression are still not exactly understood.

Tumor tissue specimens as well the healthy colon tissue and the tissue surrounding the tumor were obtained from 50 primary colorectal cancers. The concentration of TBARS in the homogenate was determined by spectrophotometric method by Andreeva et al. AOPP concentrations in the tissue was measured by the spectrophotometric method by Vitko et al. Catalase activity in plasma was determined by spectrophotometric method by Goth.

TBARS and AOPP levels were significantly higher in the tumor tissue compared to the control healthy tissue ($p<0.001$). Also, the tissue surrounding the tumor had higher concentration of TBARS and AOPP compared to the control healthy tissue ($p<0.001$). The activity of catalase in tumor tissue was significantly lower in comparison to the healthy colon tissue ($p<0.001$).

This study defines that colorectal carcinogenesis is associated with serious oxidative stress and proves the involvement of lipid peroxidation and oxidative modification of proteins in malignant process and the spread of lipid peroxidation from malignant into the adjacent non-malignant colon tissue. The results also show a lower activity of catalase confirming the relevance of oxidative-antioxidative disorders. *Acta Medica Medianae 2016;55(3):32-37.*

***Key words:*** colon carcinoma, TBARS, AOPP, catalase

University of Niš, Faculty of Medicine, Niš, Serbia[1]
Clinical Center Niš, Niš, Serbia[2]

*Contact:* Andrej Veljković
Blv. Zorana Đinđića 71, 18 000 Niš, Serbia
Email: veljkovicandrej@yahoo.com

## Introduction

Colorectal cancer is one of the most frequent neoplastic diseases in the human population, and one of the most frequent causes of death, with approximately 140.000 new cases and 56.000 cancer deaths each year (1). The starting event responsible for the transformation of normal cells of the colonic mucosa into neoplastic cells still has not been completely clarified.

There are a lot of pathological factors, including reactive oxygen species (ROS) involved in the process of cancer initiation and progression (2). Hypermethylation of the gene promoter region (thereby blocking transcription) and oxidative damage to nuclear DNA (oxidative stress) are the two main mechanisms in the initial stages of colorectal carcinogenesis (3, 4). An increased formation of reactive oxygen species in the intestinal lumen and continuous exposure of the mucosa to these free radicals promote oxidative damage to the DNA of the epithelial cells, thereby triggering the appearance of genetic mutations (3). When these mutations harm the genes responsible for controlling the cell cycle or the DNA repair system, cell clones with proliferative autonomy can emerge, thereby representing the initial mechanism for colorectal carcinogenesis.

In addition, information on the biochemical alterations in tissue and the tissue surrounding the tumor, its correlation with the clinical staging of the disease, is lacking.

It is known that ROS are formed in excess in chronic diseases of the gastrointestinal tract (5), but the precise mechanisms of oxidative stress being induced in cancer cells and the role of ROS

in colorectal cancer progression are still not exactly understood. Changes in some parameters of antioxidative system in colorectal cancer were found in some earlier studies (6).

In the pathology of oxidative stress, the main process is often lipid peroxidation, which depends a lot on the dietary factors. A case control study of colorectal cancer in a multiethnic population suggests that the ratio of polyunsaturated to saturated fat may be a better indicator of colorectal cancer risk than the absolute amount of specific fats in the diet (7).

Persistent oxidative stress in colorectal carcinoma patients with decreased concentration of antioxidant vitamins together with a lower amount of uric acid may be responsible for the formation of pro-oxidative environment in these patients (8).

**Aim**

The aim of this study was to determine the level of oxidative stress using the concentration of lipid peroxidation products such as TBA reactive substances (TBARS) and advanced oxidation protein products (AOPP) in colon cancer tissue and in the surrounding colon tissue compared to the healthy tissue that served as a control. Moreover, we analyzed the changes in the activity of antioxidative enzyme catalase (CAT).

**Material and Methods**

Investigations were conducted in 50 primary colorectal cancers in I, II, III and IV clinical stages of the disease. Tissue specimens used for this study were obtained after colon carcinoma resection. The patients had received neither chemotherapy nor radiation therapy before tumor resection. As a control, the same amount of samples was collected from macroscopically unchanged colon regions of the most distant location from the cancer, at least 15cm from malignant colon tissue. Specimens of the colon mucosa were also taken from the tissue immediately surrounding the tumor with no pathochistological manifestations of carcinogenesis.

Preparation of tissue samples

Tissues were removed quickly and placed into iced 0.15 mol/L NaCl solution, perfused with the same solution to remove blood cells. Next, the tissue samples were blotted on a filter paper, weighed and homogenized under the standardized conditions; 10% homogenates were frozen at - 20 oC and kept until assayed.

Biochemical assays

The concentration of TBARS in the homogenate was determined using the spectrophotometric method by Andreeva et al. The concentration is expressed in μmol/l (9).

AOPP concentrations in the tissue were measured by the spectrophotometric method by Witko et al. The concentration is expressed in μmol/mg of chloramine T (10).

Catalase activity in plasma was determined using the spectrophotometric method by Goth, which is based on the ability of catalase to break down the substrate (H2O2). Enzyme activity was expressed in catalytic units per liter of serum (kU/L) (11).

The modified cell protein content was determined by the method of Lowry et al., using the bovine serum albumin as a standard (12).

Statistical analysis

The results were expressed as mean±SD. Statistical analysis was performed using the Student's t-test and ANOVA post hoc test, reformed by means of commercially available statistics software package (SPSS® for Windows, v. 9.0, Chicago, USA). Statistical significance was set to $p<0.05$.

**Results**

TBARS levels in the tumor tissue were significantly higher in comparison to the healthy colon tissue ($p<0.001$). Also, the tissue surrounding the tumor had higher concentration of TBARS compared to the control healthy tissue ($p<0.001$). (Figure 1).

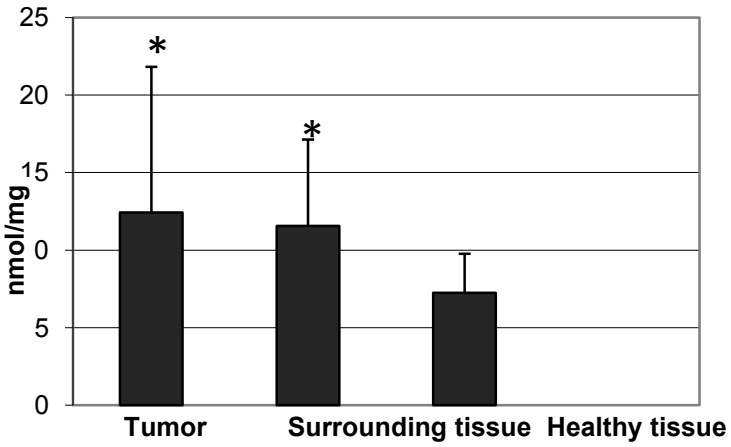
The level of AOPP in the tumor tissue was significantly higher in comparison to the healthy colon tissue ($p<0.001$). Also, the tissue surrounding the tumor had higher concentration of AOPP compared to the control healthy tissue ($p<0.05$). (Figure 2).

Activity of catalase in the tumor tissue was significantly lower in comparison to the healthy colon tissue ($p<0.001$). In addition, the tissue surrounding the tumor had lower activity of catalase compared to the control healthy tissue, but without statistical significanse. (Figure 3).

**Discussion**

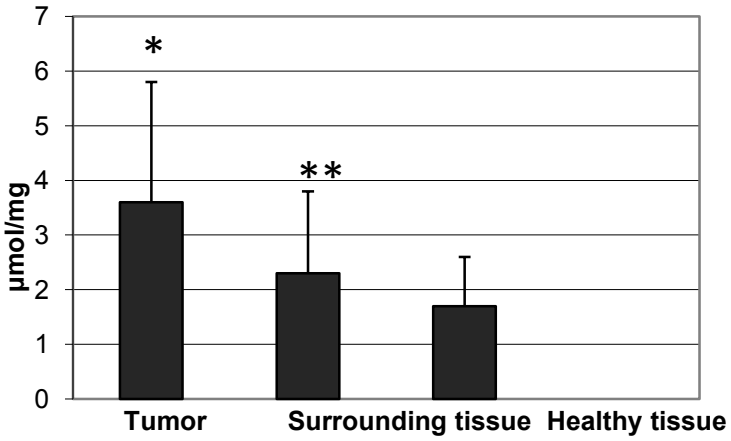
The pathophysiology of colorectal cancer, which is one of the most common carcinomas in humans, is still under investigation. One of the potential explanations is that colorectal cancer is initiated by environmental genotoxic agents causing cellular overproduction of reactive oxygen species (ROS). As a consequence, extensive oxidative stress can cause genetic alterations required for neoplastic progression and lead to a cycle of disrupted cell death and regeneration (13). There is a great body of evidence that shows that oxidative stress plays an important role in the molecular mechanism of colorectal cancer (14, 15).

Oxygen radical production, which increases with clinical progression of diseases, involves in-



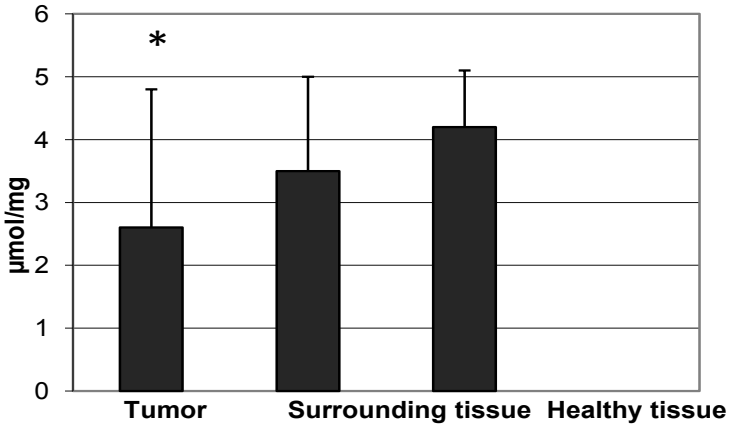

* p<0.001 vs healthy tissue

**Figure 1**. TBARS levels in tumor tissue, tissue surrounding the tumor and healthy tissue




* p<0.001 vs healthy tissue
** p<0.05 vs healthy tissue

**Figure 2**. AOPP levels in tumor tissue, tissue surrounding the tumor and healthy tissue




* p<0.001 vs healthy tissue

**Figure 3.** Catalase activity in tumor tissue, tissue surrounding the tumor and healthy tissue

creased lipid peroxidation, as a result of which cellular membrane degeneration and DNA damage ensue. The extent of lipid peroxidation could be determined by estimating the final lipid peroxidation products-TBARS and AOPP compounds known to produce protein cross-linking through the Schiff's base with DNA and DNA damage (16).

The results of our investigation show that colon cancer tissue has a significantly higher concentration of TBARS and AOPP compared to the healthy colon tissue that served as a control. The tissue surrounding the tumor also has a higher concentration of these oxidative products compared to control, and oxidative stress may be included in the process of tumor development and local invasion.

The formation of reactive oxygen species is a normal consequence of a variety of essential biochemical reactions. It is also known that oxygen radicals are formed in excess in chronic diseases of the gastrointestinal tract (17). The main source of oxidants in the gut is probably the phagocytes, which are accumulated in the mucus of patients with bowel diseases, and could generate oxidants upon activation, which might contribute to the increased risk of cancer (18). Some of the cytokines, as the resulting process of phagocytosis, can produce large amounts of ROS (19). It has been shown that elevated plasma level of TNF is responsible for increased oxidative DNA damage of CD 34 cells (20).

The results of our assays are in accordance with the results of Skrzydlewska et al. (21) and Lauschke et al. (22) who even shown that lipid peroxides can be used as a promising additional marker in patients with colon cancer. Also, Hendrickse et al. (23) as well as Ozturk et al. (24) showed a concomitant increase of levels of malondialdehyde (MDA) in patients with colorectal cancer, which is a sensitive marker of radical mediated lipid peroxidation, which has been found systemically as well as in tissue samples of cancer.

Kondo et al. proposed that colorectal carcinoma, but not adenoma cells, are exposed to more oxidative stress than the corresponding non-tumorous epithelial cells, regardless of clinical stage and histology, and that oxidative stress in carcinoma cells might stimulate cellular proliferation (25).

In this process, the final products of lipid peroxidation, as well as other products resulting from polyunsaturated fatty acid damage, could cause protein breakdown (26). Lately, several investigators have focused their studies on structural protein modifications by free radicals (27). It has been demonstrated that superoxide anion as well as alkoxyl peroxyl and radicals could inactivate one of the antioxidant enzymes-catalase and reduce the effectiveness to defend against free radical damage (28). In this paper, it was shown that during colorectal cancer development, the activity of catalase decreased. The lowest activity of this antioxidative enzyme is in the tumor tissue. Catalase is used by cells to defend against the toxic effects of hydrogen peroxide, which is generated by numerous reactions and/or environmental agents or more often by the action of superoxide dismutase, enzymes while detoxifying superoxide anion (29).

Since catalase activity reduced, the level of hydrogen peroxide increases in cancer tissue. It may be linked with the report which showed that some human cancer lines produced a large amount of hydrogen peroxide (30). At the same time, oxygen radicals might increase the secretion of the matrix metalloproteinase and collagenase as well as the production of angiogenic factors (e.g. VEGF and IL-8). These factors could promote not only the local growth of neoplasm but also metastasis (31). Oxidants, including hydrogen peroxide, have been found to be able to induce the expression of gene-coding enzymes of the antioxidative system. This kind of induction of antioxidative endogenic enzymes caused by hydrogen peroxide was found in the human fibroblast cultures (32). This increase might be caused by more extensive accessibility of enzymatic cofactors such as transient metal ions (33).

As a result of oxidative stress, iron and copper ions would become more accessible to antioxidative enzymes.

## Conclusion

This study defines that colorectal carcinogenesis is associated with serious oxidative stress and proves the involvement of lipid peroxidation and oxidative modification of proteins in malignant process and the spread of lipid peroxidation from malignant into the adjacent non-malignant colon tissue. The results also show a lower activity of catalase, confirming that the advancement of oxidative-antioxidative disorders is followed by the progression of colorectal cancer.

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# Parametri oksidativnog stresa u tkivu karcinoma kolona

*Andrej Veljković[1], Goran Stanojević[1,2], Branko Branković[1,2], Dušica Pavlović[1], Ivana Stojanović[1], Tatjana Cvetković[1,2], Tatjana Jevtović[1], Dušan Sokolović[1], Jelena Bašić[1], Milena Despotović[1], Branka Đorđević[1], Gordana Kocić[1]*

Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija[1]
Klinički centar Niš, Srbija[2]

Kontakt: Andrej Veljković
Bulevar Zorana Đinđića 71, 18 000 Niš, Srbija
E mail: veljkovicandrej@yahoo.com

Kolorektalni karcinom je jedno od najčešćih malignih oboljenja u ljudskoj populaciji i jedan od najčešćih uzroka smrti. Reaktivne vrste kiseonika (RVK) su uključene u procese inicijacije i progresije tumora. Poznato je da se RVK prekomerno formiraju kod hroničnih bolesti gastrointestinalnog trakta, ali precizan mehanizam uticaja oksidativnog stresa na kancerogenezu i njegova uloga u progresiji tumora nije još uvek razjašnjena. Uzorci tkiva karcinoma, kao i zdravog tkiva, ali i tkiva koje okružuje tumor, uzeti su od 50 bolesnika sa primarnim tumorom debelog creva. Koncentracija TBA-reagujućih supstanci (TBARS) u homogenatu određivana je metodom po Andreevoj i sar. Koncentracija uznapredovalih oksidacionih produkata proteina (AOPP) merena je spektrofotometrijskom metodom po Vitku i sar. Aktivnost katalaze je određivana spektrofotometrijskom metodom po Gotu.

Nivoi TBARS i AOPP su statistički signifikantno veći u tumorskom tkivu u poređenju sa kontrolom ($p<0,001$). Takođe, tkivo koje okruzuje tumor je imalo veću koncentraciju TBARS i AOPP u poređenju sa kontrolom ($p<0,001$). Aktivnost katalaze u tumorskom tkivu je značajno manja u poređenju sa zdravim tkivom kolona ($p<0,001$).

Ova studija pokazuje da je kolorektalna kancerogeneza povezana sa značajnim oksidativnim stresom i pruža dokaze o uključenosti lipidne peroksidacije i oksidativne modifikacije proteina u malignom procesu i širenju lipidne peroksidacije iz malignog u okolno nemaligno tkivo kolona. Rezultati, takođe, pokazuju smanjenu aktivnost katalaze, dokazujući značaj oksidativno-antioksidativnih poremećaja. *Acta Medica Medianae 2016;55(3):32-37.*

***Ključne reči:*** karcinom kolona, TBARS, AOPP, katalaza