Colorectal cancer is still a major medical, economical and public health problem. Pathogenesis of colorectal cancer remains unknown. It is thought both genetic and environmental factors contribute to the etiology and progression of the disease. Reactive oxygen species are known to play a dual role in biological systems: they can be either harmful or beneficial. Oxygen-free radicals are important mediators of damage to cell structures, including lipids, proteins and nucleic acids. Radical-related damage to cell structures has been proposed to play a key role in the development of many diseases including cancer.

Humans have evolved complex antioxidant strategies to protect cells from oxidation. Total antioxidant capacity (TAC) considers the cumulative action of all the antioxidants present in plasma or other body fluids.

**The aim of the study** was to investigate antioxidant status in patients with colorectal cancer measuring plasma TAC as a tool.

**Material and methods.** The study group comprised 102 patients in different clinical stages operated on for colorectal cancer. To evaluate plasma total antioxidant capacity we used “Total Antioxidant Status Kit” – Randox

**Results.** Statistical evaluation of results demonstrated significantly lower serum total antioxidant capacity in patients with colorectal cancer, as compared to the healthy control group. We observed increase mean plasma total antioxidant capacity correlating with decrease of clinical disease stage.

**Conclusions.** Colorectal patient have impairment antioxidant barrier. The deterioration of its functioning corresponds with the stage of the disease.

**Key words:** colorectal cancer, antioxidant barrier, oxygen-free radicals

Colorectal cancer remains a major medical, economical, and public health problem. It is one of the most common malignant neoplasms diagnosed worldwide. Currently, it is considered as the second most frequent malignant neoplasm, regardless patient gender. Mortality connected with colorectal cancer in male patients is second most frequent, while in female patients-third (1).

Early diagnosis of rectal cancer is connected with a 90% recovery rate. Unfortunately, in Poland 60-70% of colorectal cancer cases are diagnosed in stages III and IV, thus, poor treatment results (2).

The pathogenesis of colorectal cancer remains unknown. In spite of the fact that for tens of years much attention is directed towards the analysis of risk factors and different
hypotheses of the development of the disease, numerous questions remain unanswered (3). Epidemiological factors according to many authors are responsible for most cases (80-90%) of colorectal cancer (4). A significant role is attributed to the type of nutrition. Diets are considered as one of the main, modifiable factors influencing the occurrence of colorectal cancer in human beings (5). Nutritional elements can not only lead towards intestinal cell structure damage predisposing the development of cancer, but also exert a protective effect.

In most cases colorectal cancer develops on the basis of an adenoma in patients without genetic risk factors (6, 7).

It is well-known that oxygen in particular cases can be toxic. During the past 30 years intensive investigations have been undertaken concerning the metabolic role of oxygen, which would explain the mechanisms of its toxicity. In 1939, Michaelis presented a hypothesis, which was confirmed by future investigations demonstrating that cellular oxidation and reduction processes progressed by means of free-radical stages with the creation of reactive oxygen species.

Basic biochemical processes, such as cellular oxidation and nucleotides changes are the source of these reactive oxygen species. Apart from the physiological cellular metabolism, the biological activity of many exogenous chemical substances (combustion gases, industrial smoke, drugs), and physical factors (ionizing, thermal and ultraviolet radiation) are responsible for the development of free-radical particles. There increased synthesis is also observed in case of phagocytosis during the course of an infection, as a consequence of an injury, as well as inflammatory condition (8, 9).

Free oxygen radicals can react with all cellular substances (proteins, lipids, carbohydrates, and nucleic acids). Under their influence, in case of proteins, one can observe protein chain structure damage, oxidation of residual aminoacids, and modification of prosthetic groups. This can lead towards rupture of the polypeptide chain, development of cross-bonds and changes in the structure of residual aminoacids and non-aminoacid elements of complex proteins (10). This in turn leads towards damage or loss of protein function (antigen property changes, enzymatic activity inhibition).

Lipid peroxidation is one of the best known free-radical biological processes. Lipid peroxidation products have the ability to modify the physical properties of cellular membranes, disturbing their two-layered organization, which leads towards membrane permeability changes and there depolarization. Thus, one can observe intracellular membrane integral loss (11).

The influence of reactive oxygen species directly on the genetic material of the cell leads towards nucleic acid structural changes, DNA strand rupture, as well as replication and transcription disturbances. If damage is not fixed and concerns genes responsible for the regulation of apoptosis processes, protooncogenes or suppressor genes, the above-mentioned can initiate disturbances leading towards improper proliferation, disturbed cellular differentiation and carcinoma development (12).

Stationary concentrations of reactive oxygen species depend on the dynamic equilibrium between the speed of their preparation and neutralization. The increased number of reactive oxygen species, systemic exposure to their additional sources or impaired functioning of the so-called “antioxidative barrier”, leads towards prooxidative-antioxidative homeostasis disturbances. The increased number of reactive oxygen species is called “oxidative stress”.

It is considered that „oxidative stress” underlies or is observed in the course of many diseases including neoplasms, such as colorectal cancer (13).

Aerobic organisms are equipped with a complex defense system protecting them from the harmful effects of reactive oxygen species. The system balance between developmental processes and elimination of reactive oxygen species is possible, thanks to the enzymes, low-molecular compounds, and selected microelements of the “antioxidative barrier”.

All oxidants serve the same function- they prevent uncontrolled, free-radical oxidation reactions. The indication of the contents and function of particular antioxidative barrier elements would prove difficult and laborious, if at all possible. The effect of all antioxidants is not the simple sum of the activity of particular elements. The total antioxidative capacity (TAC) is the measure of all components of the antioxidative barrier. TAC describes the
The antioxidative barrier in patients with colorectal cancer

Antioxidative properties of complex biological systems (serum) often better than the concentration of all antioxidants measured separately (14). Trolox, a synthetic, water-soluble vitamin E analogue is used to determine the total antioxidative capacity.

Experimental investigations ongoing for many years confirmed the influence of reactive oxygen species in the etiopathogenesis of colorectal cancer, as well as the role of the antioxidative barrier in the protection of the organism against negative oxygen compounds. The demonstration of increasing antioxidative barrier functioning disturbances, together with the stage of the neoplasm (colorectal carcinoma) would be evidence confirming the legitimacy of the use of neoplastic chemoprevention by means of antioxidant substances.

The aim of the study was to determine the antioxidative barrier in patients with distal colorectal cancer, based on the total antioxidative capacity.

MATERIAL AND METHODS

The study group comprised 102 patients (42 women and 60 men; average age- 64.4 ± 10.9 years) with colorectal adenocarcinoma operated at the Department of General and Colorectal Surgery, Medical University in Łódź, during the period between 2006 and 2008. Table 1 presented patient demographics. The investigated patient groups, depending on the stage of the disease showed no statistically significant differences, considering average patient age and gender. Twenty healthy volunteers comprised the control group. None of the investigated patients received vitamin preparations six months before study initiation.

The total antioxidative capacity (TAC) was determined using the „Total Antioxidant Status Kit“, manufactured by Randox. Absorbance measurements were performed using the Lambda 14p device (Perkin – Elmer).

Clinical diagnosis was determined on the basis of the following scheme:
- History and physical examination
- Colon examinations (rectoscopy or colonoscopy with sample collection, and their histopathological evaluation, transrectal ultrasound)
- Imaging examinations in search of distant metastases (abdominal ultrasound and CT, chest x-ray).

RESULTS

Table 2 and figure 1 presented total antioxidative capacity measurement values (Trolox equivalent in mmol/l) in patients with distal colorectal cancer, depending on the stage of the disease, as compared to the control group.

A statistically significant lower total antioxidative capacity was observed in all stages of the neoplastic disease, as compared to the control group (p<0.05). Additionally, analysis comprised the comparison between results obtained from particular disease progression

<table>
<thead>
<tr>
<th>Dukes classification</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>34</td>
<td>17</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>Mean age ±SD</td>
<td>63.1±12.8</td>
<td>65.9±9.4</td>
<td>65.9±9</td>
<td>62.6±10.2</td>
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<tr>
<td>Female:male</td>
<td>15:19</td>
<td>6:11</td>
<td>12:18</td>
<td>8:13</td>
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<table>
<thead>
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<th>TAC</th>
<th>Dukes classification</th>
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<tbody>
<tr>
<td>Median</td>
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<td>B</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>0.155</td>
<td>0.139</td>
</tr>
<tr>
<td>Arithmetical mean</td>
<td>1,191</td>
<td>1,070</td>
</tr>
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</table>
(stage) groups. We observed a decrease in the total antioxidative capacity, in relationship to the stage of the disease. However, in case of stages C and D these differences proved statistically insignificant.

DISCUSSION

Epidemiological investigations suggest that the diet rich in antioxidative vitamins can significantly reduce the risk of cancer development, including colorectal carcinoma (15). Although the practical introduction of chemoprevention requires further investigations, it is only a matter of time when we will prove its efficiency. The evaluation of the condition of the antioxidative barrier during consecutive stages of the neoplastic disease, and determination of its increasing impairment (disease progression) would be evidence of the significant role of reactive oxygen species in the pathogenesis of neoplastic diseases. Thus, the legitimacy of chemoprevention in case of the above-mentioned diseases with the use of antioxidative activity substances.

The measurement of the total antioxidative capacity, considering the condition of the antioxidative barrier has had for many years a strong position in literature data. Several methods considering the measurement of the above-mentioned parameter were proposed. The most commonly used methods are based on the measurement of the inhibition of the oxidative reaction of the indicator substance. One of the most common and classical methods was that proposed in 1993 by Miller and Rice-Evans (16). The main advantage of this method is the possibility to compare results obtained by different research centers, since the conditions of these measurements are clearly determined, and standardized reagents can be easily purchased.

The results of our investigations demonstrated antioxidative barrier functioning disturbances in case of a patient with distal colorectal cancer evaluated on the basis of total antioxidative capacity measurements. In case of every stage of the neoplastic disease (according to Dukes classification), as compared to the control group, we observed a statistically significant smaller total antioxidative capacity (TAC). The obtained results gained confirmation in light of the analysed medical literature. Plavec et al. demonstrated in animal models with different neoplasms, such as lymphomas, sarcomas, and melanomas (17), decreased total antioxidative capacity values. Hietanen et al. obtained lower TAC values in patients with diagnosed breast, colon and prostate cancer (18), while Erhola et al. received similar results in case of 13 patients with lung cancer. The above-mentioned correlated with the simultaneously determined oxidative parameters of protein modification (19). The continuous decrease of the total antioxidative capacity with the progression of the neoplastic disease (colorectal cancer) was observed by Skrzydlewska et al. (20), and Czeczot et al. (21). The increasing antioxidative barrier disturbances with neoplastic progression were also observed by Kim et al. in case of cervical cancer (22) and by Czeczot et al. in case of hepatocellular carcinoma (23). Balcerska et al. determined the elements of the antioxidative barrier in children during treatment of different stages of the neoplasm: early, remission, disease progression, and five years after therapy. In case of the initial period of the disease, one observed significant reduction of the activity of the antioxidative barrier. Improvement of the patients’ condition and clinical remission of the neoplastic disease was accompanied by an increase of the antioxidative activity (24). Similar dependencies were not observed by Mantovani et al. (25). The latter, observed statistically smaller TAC values in 81 patients with different stages of the neoplastic disease (II-IV), as compared to the control group. However, the above-mentioned authors observed no correlation between the degree of antioxidative barrier functioning disturbances and the stage of the disease. The analysed patient population is heterogeneous.
comprising patients with 17 different neoplasms, which in my opinion significantly impedes the interpretation of results.

Colorectal cancer remains a major challenge, not only in the field of diagnostics and therapy, but above all in case of prophylaxis. Forecasts predict a doubling of the number of malignant neoplasms within the next twenty years (26). One way to improve this condition is research aimed at establishing measures confirmed of preventive action. The process of oncogenesis, which usually lasts 10–15 years, could successfully become the target of non-specific prophylactic activity–chemoprevention with the use of antioxidative activity measures (27).

In spite of the lack of clear study results (28) concerning the use of antioxidative vitamins in the prevention of neoplasms, there is substantial agreement amongst influential institutions as to the advisability of recommending a diet rich in natural products containing antioxidative vitamins, as an element of neoplastic disease prophylaxis (29). Our results also suggest the usefulness of substances of antioxidative character in the widely understood chemoprevention of colorectal cancer.

REFERENCES

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