Analysis of Oxidative Stress Indicators in Polish Patients With Prostate Cancer

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Abstract

**Aims:** The aim of the study was to analyze the activity of antioxidant enzymes (glutathione S-transferase, catalase, superoxide dismutase) in order to determine the role of detoxification mechanisms in prostate cancer. The concentration of malondialdehyde, which is an indicator of lipid peroxidation in cancer patients, was also tested.

**Methods:** The activities of superoxide dismutase (SOD), catalase CAT and glutathione S-transferase (GST) were measured using ready-made kits; lipid peroxidation intensity was determined by the thiobarbituric acid method.

**Results:** Superoxide dismutase was the only enzyme among antioxidant and detoxification enzymes for which a statistically significant difference in activity was found between the studied groups [1.4 U * ml⁻¹ in patients vs. 1.6 U * ml⁻¹ in control]. No statistically significant differences were found for two other biomarkers of antioxidant activity (GST, CAT). There were also no statistically significant differences in the concentration of MDA between the group of men with prostate cancer and the control group.

**Conclusion:** The lower SOD activity in men with prostate cancer may be due to a deficiency in their antioxidant defense system.

Introduction:

In recent years, the oxidative stress (in a broad sense) has been the subject of many studies. It is caused by the overproduction of molecules commonly known as "free radicals", more broadly referred to as reactive oxygen species (ROS). ROS include, among others, hydroxyl radical (* OH), superoxide anion (O²⁻), singlet oxygen and hydrogen peroxide (H₂O₂), which as an exception does not have an unpaired electron. These molecules are formed during the body's physiological processes, such as aerobic respiration or inflammation. They are involved in many cellular processes, including the secretion of hormones, the functioning of the immune system, muscle contractions, apoptosis, vascular tone regulation and the elimination of xenobiotics from the body (Czajka 2006).

Healthy organism has defenses which detoxify reactive oxygen species by complex antioxidant mechanisms. They are especially important when the production of ROS increases, which is a consequence of smoking, drinking alcohol, improper diet, excessive physical stress, exposure to environmental pollution or ionizing radiation. Excessive production of free radicals, due to their high reactivity, can have a very negative effect on the body. The consequences of the uncontrolled action of reactive oxygen species on cells include oxidation of cell membranes, modification of proteins, and changes in the structure of DNA that may cause mutations and, ultimately, lead to initiation of the neoplastic process. Such an intensified attack of free radicals on the body's structures, when the antioxidant defense mechanisms fail and the physiological concentrations of ROS are exceeded, is called oxidative stress (Czajka 2006, Valko et al. 2006, Halliwell 2007, Visconti et al. 2009).
Defense mechanisms maintain an adequate level of free radicals so that they do not interfere with the proper functioning of the body. The formation and action of reactive oxygen species is counteracted by both enzymatic and non-enzymatic components of the antioxidant defense. Non-enzymatic mechanisms (the so-called free radical scavengers) are considered to be supplementary elements, while antioxidant enzymes seem to play a major role in the whole process (Wielkoszyński et al. 2007).

Enzymatic defenses against ROS include a system of specialized enzymes that prevent and remove free radicals. These enzymes are related to each other, participating in a cascade of events aimed at neutralizing free radicals. The most important antioxidant enzymes include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione S-transferase (Wielkoszyński et al. 2007). These enzymes cooperate in the direct neutralization of free radicals, inhibition of lipid peroxidation, reactivation of non-enzymatic elements of antioxidant defense, repair of damaged molecules and destruction of structures that could not be repaired (Wielkoszyński et al. 2007).

**Superoxide dismutase**

Superoxide dismutase is the body’s main defense mechanism against the toxic effects of peroxides. It catalyzes the decomposition of superoxide anions to hydrogen peroxide and molecular oxygen.

MnSOD is believed to be one of the most important enzymes in cell defense against oxidative stress. Mitochondria, whose DNA is highly susceptible to attack by free radicals, must be protected by an effective manganese superoxide dismutase mechanism. A disturbance in the enzyme’s activity could expose the cell to an intensified attack of ROS, which could damage the genetic material, leading to mutations, energy deficit and, consequently, the initiation of carcinogenesis (Mruk et al. 2002, Skrzycki et al. 2005, Czajka 2006, Wielkoszyński et al. 2007).

It is believed that there is a relationship between the activity of superoxide dismutase and the development of neoplastic changes in humans. The lack or limited activity of this enzyme can lead to mutations. An increase in SOD activity is associated with cancer progression and malignant transformation of neoplastic cells. On the other hand, the increased activity of SOD, which reduces the concentration of the superoxide radical, is associated with the suppression of the neoplastic phenotype. Understanding the exact mechanisms of SOD activity in healthy people and cancer patients may result in new treatment and diagnostic options in the future (Mruk et al. 2002, Skrzycki et al. 2005).

**Catalase**

Catalase is the main line of defense against highly reactive hydrogen peroxide and is involved in its decomposition into water and oxygen. The enzyme exhibits catalase activity at high concentrations of hydrogen peroxide, causing it to decompose. In turn, at a low concentration of H$_2$O$_2$, catalase shows peroxidase activity when participating in the oxidation of compounds such as methanol, ethanol, formates, nitrites or quinones (Putnam et al. 2000, Czajka et al. 2006, Šcibior et al. 2006).
An effective action of catalase is especially important in the metabolism of erythrocytes, which function at high oxygen concentrations and are therefore exposed to oxidative stress. Moreover, catalase, by converting hydrogen peroxide, does not generate additional free radicals, which protects cells against other reactive oxygen species. Oxygen from the decomposition of $\text{H}_2\text{O}_2$ can be further used in other metabolic processes (Kirkman et al. 1984, Ścibior et al. 2006).

Catalase, as an enzyme that protects cells against the toxic effects of hydrogen peroxide, is associated with mutagenesis, carcinogenesis, inflammation and protection against apoptosis. Low activity of catalase was found in patients with pneumonia, atherosclerosis, diabetes, neurodegenerative diseases, nephritis and cancer. It is believed that the reduction of the enzyme activity may be caused by prolonged exposure of patients' cells to oxidative stress. Particularly low values of catalase were observed in patients with cancer of the lung, gastrointestinal tract, kidney, breast or with leukemia (Ścibior et al. 2006).

Glutathione S-transferase

Glutathione S-transferases are ubiquitous multifunctional enzymes that play a major role in cell detoxification (Krajka-Kuźniak 2007, Wielkoszyński et al. 2007).

The main function of glutathione S-transferase is related to the participation in the second phase of detoxification of xenobiotics. The enzyme protects cells by catalyzing the conjugation of glutathione with toxins, thereby neutralizing their electrophilic sites and producing a more water-soluble products. Glutathione conjugates are further metabolized to mercapturic acid and then excreted (Cotton et al. 2000, Strange et al. 2000). For example, highly toxic and carcinogenic lipid peroxidation products such as 4-hydroxy-2,3-nonenal and other carcinogens, anti-cancer drugs, pesticides and herbicides can be detoxified according to the above mechanism. GST can also deactivate oxidative stress products, such as quinones, hydroperoxides, α- and β-unsaturated carbonyls (Krajka-Kuźniak 2007).

GSTs are enzymes that reduce the harmfulness of xenobiotics, improve their solubility in water and, consequently, facilitate their excretion from the body. These enzymes are associated with susceptibility to diseases caused by toxic extracorporeal compounds, including cancer (Wielkoszyński et al. 2007).

Malondialdehyde as an indicator of oxidative stress

Reactive oxygen species participate in the free radical oxidation of unsaturated fatty acids in lipids, i.e. in the so-called lipid peroxidation (Gawel et al. 2004).

The end products of lipid peroxidation can be low molecular weight three-carbon malondialdehyde (MDA), and other aldehydes and hydroxyaldehydes. MDA is one of the most mutagenic products of lipid peroxidation. It reacts with DNA to form premutagenic lesions (Przybyszewski et al. 2005, Krzystak et al. 2009, Kulbacka et al. 2009).
Elevated levels of free radicals boost lipid peroxidation and increase the production of MDA. It is believed that the content of malondialdehyde may be an indicator of increased oxidative stress and the body antioxidant status (Gawel et al. 2004, Kulbacka et al. 2009). Elevated levels of MDA in the blood were found in patients with breast, colorectal or prostate cancer (Surapaneni et al. 2006).

Oxidative stress in prostate cancer

The etiopathogenesis of neoplastic diseases is still the subject of many scientific studies, including the analysis of the imbalance between oxidation and reduction (Kaya et al. 2017). It has long been known that oxidative stress, including that caused by environmental factors, increases the activity of antioxidants that contribute to intracellular redox homeostasis. When this action is disturbed, an organism without adequate defense may be exposed to damage, including changes in the genetic material resulting in carcinogenesis (Agarwal et al. 2006, Kaya et al. 2017).

Currently, the drastic increase in cancer incidence is a growing problem in developed and developing countries. It is related to factors such as environmental pollution, diet and smoking. Today, cancer is the leading cause of death in developed countries. Prostate cancer is the third most frequently diagnosed neoplastic disease, after lung cancer and colorectal cancer (Farhood et al. 2018, Religioni et al. 2020). According to data from 2016, prostate cancer is the most common cancer in men in Poland and the third cause of cancer-related death in men. The overall mortality rate due to this disease slightly exceeds the European average (according to data from 2013, the mortality rate in Poland was 12.4/100,000, and 12.1/100,000 in the entire EU) (Religioni et al. 2020).

As a part of the research on the prostate cancer etiopathogenesis (which has not yet been clearly explained), the mechanisms of inactivation and excretion of toxic xenobiotics and harmful substances produced by the body itself were investigated. To determine the importance of antioxidant mechanisms in prostate cancer, the activity of enzymes such as catalase (CAT), superoxide dismutase (SOD) and the concentration of glutathione S-transferase (GST) were tested (Agarwal et al. 2006, Arsova-Sarafinovska et al. 2009, Battisti et al. 2011, Freitas et al. 2012). Battisti et al. (2011) demonstrated reduced catalase activity and increased superoxide dismutase activity in patients with prostate cancer compared to healthy controls. Studies involving patients from Macedonia and Turkey (Arsova-Sarafinovska et al. 2009) showed decreased catalase activity, as in the previously cited study. However, the activity of superoxide dismutase was lower in patients with prostate cancer than in the control group. The analysis of the influence of oxidative stress on prostate cancer cells by Freitas et al. (2012) included the measurement of the concentration of glutathione S-transferase. It was shown that the concentration of GST in cells treated with hydrogen peroxide was significantly lower, which might indicate a relationship between the low level of glutathione S-transferase and the progression of neoplastic changes. So far, inconclusive data indicate an imbalance of antioxidants in patients with prostate cancer, supporting the hypothesis of the influence of oxidative stress on this type of cancer (Arsova-Sarafinovska et al. 2009, Battisti et al. 2011, Freitas et al. 2012).
The aim of the study was to analyze the activity of antioxidant enzymes (glutathione S-transferase, catalase, superoxide dismutase) in order to determine the role of detoxification mechanisms in prostate cancer. The concentration of malondialdehyde, which is an indicator of lipid peroxidation in cancer patients, was also tested.

**Materials And Methods:**

In the study we used blood samples collected from 66 patients of the Oncology and Brachytherapy Department of the Oncology Center in Bydgoszcz with diagnosed prostate cancer. Sixty four healthy volunteers were recruited at the Outpatient Clinic (SP ZOZ) in Mogilno and the Department of Prevention and Health Promotion of the Oncology Center in Bydgoszcz to serve as controls for patient group. Men with a recent blood transfusion were excluded from the cancer group. The control group consisted of men over 50 years of age who were eligible for the study if they had not been diagnosed with cancer throughout their lives, had no major surgery and, most importantly, their PSA (up to 4 ng/ml) and rectal examination were normal.

The material for analysis was blood collected from the ulnar vein. The blood collection was performed by qualified medical personnel. The material was collected in two different tubes with a total capacity of 16 ml. To analyze CAT and SOD activity and MDA concentration, 10 ml serum test tubes with clot activator were used; 6 ml tubes with lithium heparin were used for GST activity analysis. In order to obtain separated serum, blood samples were centrifuged (2000×g, for 15 min, at 4°C), and the material needed for analysis was transferred to Eppendorf tubes. All tubes with test material were stored at −80°C until the planned analyzes were performed. The study was approved by Ethical Committee of Collegium Medicum in Bydgoszcz (KB 65/2012; consent dated February 28, 2012, and the relevant annexes).

**Glutathione S-transferase activity**

Plasma glutathione S-transferase activity was determined using the standardized Glutathione S-transferase Assay Kit (Cayman Chemical Co. Item No. 703302). The analyzes were performed on 96-well plates according to the methodology provided by the manufacturer. Three non-enzymatic background samples were prepared by placing 170 µl of Assay Buffer and 20 µl of glutathione in the wells. Then, 3 samples of the positive control containing equine liver GST were prepared by adding 150 µl of Assay Buffer, 20 µl of glutathione and 20 µl of control GST. The remaining wells were filled with 20 µl of test plasma, 150 µl of Assay Buffer and 20 µl of glutathione. The reaction was started by adding 10 µl of 1-chloro-2,4-dinitrobenzene (CDNB) to all wells in the plates. The plates were carefully shaken for a few seconds on a shaker. Five absorbance measurements were made every minute at 340 nm using a plate reader (Multiskan RC version 6.0, Labsystems). Glutathione S-transferase activity was calculated by analyzing the change in absorbance per minute corrected for non-enzymatic background. The calculated GST activity was expressed in nmol/min/ml.

**Activity of superoxide dismutase**
Serum superoxide dismutase activity was determined using a standardized Superoxide Dismutase Assay Kit (Cayman Chemical Co. Item No. 706002). The analyzes were performed on 96-well plates according to the methodology provided by the manufacturer. 200 µl of Radical Detector solution (tetrazolium salt solution) was added to the samples and 10 µl of standards. The reaction was started by adding 20 µl of xanthine oxidase solution to all wells. The plate was carefully shaken for several seconds to mix the reaction components, and then incubated on a shaker for 20 min at room temperature. The absorbance was measured at 450 nm using a plate reader (Multiskan RC Version 6.0, Labsystems).

The superoxide dismutase activity in the samples was calculated from the standard curve and expressed in U/ml.

Catalase activity

Serum catalase activity was determined using a standardized Catalase Assay Kit (Cayman Chemical Co. Item No. 707002). The analyzes were performed on 96-well plates according to the methodology provided by the manufacturer. Assay Buffer (100 µl) and methanol (30 µl) were added to the samples, to the standards and to 20 µl of bovine liver catalase which served as a positive control. The reaction was started by adding 20 µl of hydrogen peroxide to all wells. The plate was incubated on a shaker for 20 min at room temperature. To terminate the reaction, 30 µl of potassium hydroxide was added to samples, standards and positive controls, followed by 30 µl of chromogen (Purpald). The plate was then incubated on a shaker for 10 min at room temperature. Next, 30 µl of potassium periodate was added to all wells. The plate was incubated on a shaker for 5 min at room temperature. The absorbance at 540 nm was measured using a plate reader (Multiskan RC Version 6.0, Labsystems). The catalase activity in the samples was calculated from the standard curve and expressed in U/ml.

Analysis of MDA concentration

Malondialdehyde concentration, indicating the intensity of lipid peroxidation processes, was measured by the method of Rice-Evans et al. (1991) as modified by Atmaca (2004). To the analyzed serum and one of the controls containing 200 µl of distilled water the following reagents were added: 20 µl of 2% BHT (butylhydroxytoluene) in ethanol, 1 ml of 15% TCA (trichloroacetic acid) in 0.25M HCl and 1 ml of 0.37% TBA (thiobarbituric acid) in 0.25M HCl. In the second control sample TBA was replaced by 1 ml of distilled water. The samples were vortexed and heated in a water bath at 100°C for 10 minutes. After cooling, the samples were centrifuged. The absorbance in the supernatant was measured at 535 nm against distilled water as control. The obtained absorbances were corrected by subtracting the absorbances of controls with TBA replaced by distilled water. MDA concentration in the samples was calculated using the absorbance coefficient (156 mmol⁻¹cm⁻¹). The concentration was expressed in µM.

Statistical analysis

Statistical analysis was performed with STATISTICA 10 software for Windows 10 using descriptive statistics and statistical significance tests.
In the first step Shapiro–Wilk normality test was used to determine if the analyzed data were normally distributed. If the data were not normally distributed, then between-group differences were analyzed using the Mann-Whitney U test. For all analyzes a significance level of 5% (p < 0.05) was adopted.

**Results:**

GST, SOD, CAT activities and MDA concentration

Table 1 presents the parameters describing the activities of antioxidant and detoxification enzymes, and the concentration of malondialdehyde.

**Table 1.** MDA concentration and CAT, SOD and GST activities in the group of patients (n=66) and in the control group (n=64).

| Descriptive statistics |  ĥx  | SD  | Min | Q1  | Me  | Q3  | Max  |
|------------------------|------|-----|-----|-----|-----|-----|------|
| **Control group**      |      |     |     |     |     |     |      |
| MDA [μM]               | 0.4  | 0.3 | 0.1 | 0.3 | 0.3 | 0.5 | 2.1  |
| CAT activity [nmol/min/ml] | 52.6 | 38.7 | 9.6 | 27.2 | 37.7 | 69.6 | 154.6 |
| SOD activity [U/ml]    | 1.9  | 1.4 | 0.0 | 1.3 | 1.6 | 2.0 | 9.4  |
| GST activity [U/ml]    | 1.1  | 1.1 | 0.0 | 0.3 | 1.0 | 1.7 | 5.7  |

| **Group of patients**   |      |     |     |     |     |     |      |
| MDA [μM]               | 0.6  | 0.9 | 0.2 | 0.3 | 0.3 | 0.5 | 7.2  |
| CAT activity [nmol/min/ml] | 46.8 | 32.4 | 0.0 | 26.9 | 39.5 | 58.1 | 159.9 |
| SOD activity [U/ml]    | 1.4  | 0.5 | 0.3 | 1.0 | 1.4 | 1.6 | 3.0  |
| GST activity [U/ml]    | 0.9  | 0.8 | 0.0 | 0.3 | 0.7 | 1.3 | 3.0  |

The analysis of the distribution of the studied variables (MDA concentration and CAT, SOD and GST activities) using the Shapiro–Wilk test is presented in Table 2.

**Table 2.** Results of Shapiro–Wilk test performed for MDA concentration and CAT, SOD and GST activities in cancer group (n=66) and control (n=64).

Since the analyzed variables were not normally distributed, further analysis was performed using the Mann–Whitney U test. The test results for MDA concentration and the activity of antioxidant and detoxification enzymes in patients and control are presented in Table 3. A statistically significant difference in superoxide dismutase activity was found between the group of patients and the control group: SOD activity was lower in prostate cancer patients (Fig. 1). The obtained result indicates an abnormal antioxidant balance in men with prostate cancer.

**Table 3.** Comparison of MDA concentration and CAT, SOD and GST activities in patients (n=66) and control (n=64).
Shapiro–Wilk test for normality

| Analyzed variable   | Group of patients | Control |   |   |
|---------------------|-------------------|---------|---|---|
|                     | W     | p     | W     | p  |
| MDA [µm]            | 0.665 | <0.001 | 0.308 | <0.001 |
| CAT activity [nmol/min/ml] | 0.830 | <0.001 | 0.883 | <0.001 |
| SOD activity [U/ml] | 0.653 | <0.001 | 0.942 | 0.004 |
| GST activity [U/ml] | 0.843 | <0.001 | 0.881 | <0.001 |

Results of Mann–Whitney U test

| Variable                  | Sum of ranks Patients | Sum of ranks Control | Mean of ranks Patients | Mean of ranks Control | p   |
|---------------------------|----------------------|---------------------|-----------------------|-----------------------|-----|
| MDA [µm]                  | 4498.5               | 4016.5              | 68                    | 63                    | 0.415 |
| CAT activity [nmol/min/ml]| 4272                 | 4243                | 65                    | 66                    | 0.815 |
| SOD activity [U/ml]       | 3648                 | 4867                | 55                    | 76                    | 0.002 |
| GST activity [U/ml]       | 4037                 | 4478                | 61                    | 70                    | 0.184 |

Discussion:
Highly reactive free radicals that cause oxidative stress are believed to be responsible for many diseases. By reacting with components of the body's structures, they can cause DNA damage, lipid peroxidation or protein oxidation. It is difficult to determine the cause and effect relationship between disease and oxidative stress. The latter can be treated, on the one hand, as a cause and, on the other, as a result of pathological processes. Currently, attention is paid to the possible role of oxidative stress and lipid peroxidation in the development of various types of cancer (Kotrikadze et al. 2008).

Antioxidant and detoxification enzymes, i.e. catalase, superoxide dismutase and glutathione S-transferase, are biomarkers of oxidative stress in humans (Kotrikadze et al. 2008). The concentration of malondialdehyde is considered to be a reliable indicator of intensified oxidative processes and the current antioxidant status of the body (Gawel et al. 2004, Kulbacka et al. 2009). In this study, we analyzed the activity of GST (in plasma), the activity of SOD, CAT and the concentration of MDA (in serum) in patients with prostate cancer and in controls. A statistically significant difference between the groups was found only for superoxide dismutase activity. Comparison of measurements of other oxidative stress biomarkers revealed no significant differences. The median GST activity was lower in the group of patients (0.7 U/ml vs. 1.0 U/ml in the control group), but statistical significance was not reached (p = 0.184).

Superoxide dismutase is the first line of defense against the toxic effects of peroxides. Its disturbed activity allows free radicals to attack body structures, including DNA, which in turn may lead to neoplastic...
changes (Skrzycki et al. 2005, Czajka 2006). This study found reduced levels of this enzyme in people with prostate cancer compared to healthy controls. Kotrikadze et al. (2008) also reported lower SOD activity in red blood cells of patients with prostate cancer. Analysis of superoxide dismutase activity in erythrocytes of Turkish and Macedonian patients by Arsova-Sarafinovska et al. (2009) showed similar results. However, Battisti et al. (2011) showed higher SOD activity in whole blood of patients with prostate cancer compared to the control group. Similar results were reported by Surapaneni et al. (2006) who studied the activity of the enzyme in the erythrocytes of men with prostate cancer in India.

It is believed that the limited antioxidant activity of SOD may result in mutations (Skrzycki et al. 2005). Low SOD activity found in this study may be caused by disturbances in the antioxidant defense system in patients with prostate cancer (Arsova-Sarafinovska et al. 2009). Detailed interpretation of this result is hindered by the lack of significant changes in MDA concentration in patients compared to the control group. Arsova-Sarafinovska et al. (2009) showed that the decrease in SOD activity was correlated with an increased concentration of MDA, and thus with increased lipid peroxidation. This fact was explained by the exhaustion of the antioxidant defense system due to the high intensity of oxidative stress. In patients with prostate cancer included in this study, lipid peroxidation processes did not intensify compared to the controls. Therefore, a decrease in SOD activity may be associated with either a primary defect in people with prostate cancer or with impaired enzyme function as a result of disease processes. One should not expect a decrease in SOD activity as a result of long-term exposure to free radicals, because the analysis of the concentration of malondialdehyde, which is currently one of the most frequently chosen indicators of oxidative stress, did not show statistically significant differences between the groups of patients and the control group. Many cancer studies reported higher levels of MDA in patients (Kumaraguruparam et al. 2002, Polat et al. 2002, Surapaneni et al. 2006, Battisti et al. 2011), while reduced lipid peroxidation was demonstrated in studies of breast tumors (Surapaneni et al. 2006). The results obtained in this study differ from those that showed increased oxidative stress in cancer patients. However, they show that the antioxidant defense in prostate cancer patients is impaired, as manifested by reduced superoxide dismutase activity.

The activity of the other two tested antioxidant enzymes (GST, CAT) did not differ significantly from that in the control group. GST activity was lower in cancer patients, but the difference compared to the control group was not statistically significant. Also Surapaneni et al. (2006), who investigated the activity of the enzyme in plasma, did not observe statistically significant differences between the groups. In turn, the studies of Battisti et al. (2011) and Kotrikadze et al. (2008) on catalase in patients with prostate cancer showed a decrease in the activity of this enzyme. This could be a sign of enzyme depletion following prolonged elimination of free radicals. This study of the intensity of lipoperoxidation processes did not show any long-term oxidative stress in patients. In the absence of significant changes in the activity of glutathione S-transferase and catalase, it supports the hypothesis that oxidative stress is moderately intense in patients with prostate cancer.

The lower activity of superoxide dismutase in patients with prostate cancer supports the notion that the antioxidant system is disturbed in these patients. A weakened system of defense against ROS may cause
the accumulation of free radicals, which may exacerbate the neoplastic process (Skrzycki et al. 2005). Patients included in this study showed no increased lipid peroxidation and thus no increased intensity of oxidative stress. However, this result applies to the condition of patients in the course of the disease, while there is no retrospective knowledge of their condition before the development of cancer. It is possible that during the study the pro-oxidant-antioxidant balance of the patients was compensated by the treatment process and the cessation of exposure to factors inducing oxidative stress. However, a possibility of effects of free radicals other than increased lipid peroxidation or the presence of primary defects decreasing SOD activity cannot be ruled out.

Declarations

Ethics approval and consent to participate

The work was approved by Ethical Committee of Collegium Medicum in Bydgoszcz (KB 65/2012; consent dated February 28, 2012, and the relevant annexes).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

D. J.M.- Wrote the paper; Conceived and designed the analysis; Collected the data; Contributed data or analysis tools; Performed the analysis.

K. B.- Conceived and designed the analysis; Contributed data or analysis tools; Performed the analysis.

K. P.- Conceived and designed the analysis.

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**Figures**

![Box plot of SOD activity](image)

**Figure 1**

Superoxide dismutase activity in the group of patients (n=66) and in control (n=64).