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# Biochemical markers of oxidative stress in patients with inflammatory bowel diseases

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## ABSTRACT

**Introduction:** Inflammatory bowel disease (IBD) is a group of diseases of unexplained aetiology, characterized by periods of remissions and exacerbations. Reactive oxygen species (ROS) as far as disorders of balance between levels of prooxidants and antioxidants may also participate in the occurrence of IBD. The aim of the present study was an assessment of the antioxidative barrier of the organism in patients with inflammatory bowel disease.

**Material and methods:** The study group consisted of 99 patients (80 with IBD as a study group and 19 healthy as a control group) from Jan Biziel University Hospital in Bydgoszcz, Poland. Venous blood was the material for biochemical analysis: HT, GSH, GPX<sub>p</sub>, GPX<sub>RBC</sub>, GST, GR, SOD-1, MDA, NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> and CP.

**Results:** There were statistically significant differences in oxidative stress parameters observed between the study group and the control group, especially concerning HT, GSH, GPX<sub>RBC</sub>, GST, SOD-1, MDA and NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup>.

**Discussion:** The assumption that increased activity of antioxidative compounds may constitute a defence against the influence of oxidative stress may be true. Their decreased activity may participate in lowering an organism's abilities to defend against oxidative stress and cause the development of free radical diseases. Further studies into targeted preventive strategies are needed.

**Conclusions:** Prooxidative factors play an essential role in the pathogenesis of IBD. Due to the still unknown etiopathology of IBD, research on imbalances between pro-oxidants and antioxidants should be continued in larger groups of patients.

**Key words:** oxidative stress, antioxidative barrier, inflammatory bowel disease

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## Introduction

Inflammatory bowel disease (IBD) is a group of diseases of unexplained aetiology, characterized by periods of remissions and exacerbations. Two main diseases characterized by diverse etiopathology belong to this group: ulcerative colitis (CU) and Crohn's disease (CD). The main causes are genetics, improper diet, and the improper reaction of the immune system to enteric bacteria or drugs [1]. Reactive oxygen species (ROS) linked with disorders of balance between levels of prooxidants and antioxidants may also be a cause of IBD. Prevalence of the oxidative reaction causes the development of processes described as oxidative stress [2]. The result of the reaction above is

a change in the protein structure and function, oxidation of cell membranes and DNA damage [3]. The result of oxidative stress is lipid peroxidation [4], a process of oxidation of the unsaturated fatty acids, constituting elements of phospholipids. Lipid peroxidation causes the emergence of their peroxides. The main product of lipid peroxidation is malondialdehyde (MDA). MDA concentration increases if the production of ROS increases, thus cell membrane permeability changes [5].

Colonization of the large intestine is caused by a huge amount of bacteria stimulating the GALT (Gut Associated Lymphoid Tissue) system. The main task of the GALT system is discrimination between symbionts and pathologic bacteria. Normal conditions are described as a balance between intestinal microflora

and GALT functioning. The normal functioning of GALT depends on the tightness of the intercellular junctions and the mucus layer [1]. Hydrogen peroxide, produced by colonocytes, contributes to intercellular junction damage [6]. It causes permeation of bacteria to lamina propria, production of proinflammatory cytokines and stimulation of T cells [7]. Increased amounts of T cells, B lymphocytes, monocytes, mast cells and macrophages within intestinal mucosa are observed in the course of IBD. The number of polymorphonuclear leukocytes (PMN) in lamina propria also increases [1]. In particular, neutrophils, releasing significant amounts of proteases, contribute to intercellular junction unsealing, which leads to the accumulation of the aforementioned cells and penetrates the intestinal mucosa. PMNs, T cells and B lymphocytes produce ROS (such as superoxide anion, hydroxyl radical, etc.) to destroy bacteria [8]. Several enzymes take part in the process of ROS production. Superoxide dismutase (SOD-1) in the reaction of dismutation of superoxide anion leads to the production of hydrogen peroxide, which is next decomposed through catalase [9]. Glutathione peroxidase (GPX) takes part in the neutralization of hydrogen peroxide, but GPX needs reduced glutathione (GSH) for action [10]. GSH as a cosubstrate takes part in reactions of hydrogen peroxides and lipid peroxides [11]. The aforementioned reactions result in the production of glutathione disulfide (GSSG), reduced by nicotinamide adenine dinucleotide phosphate (NADPH) during a reaction catalysed by glutathione reductase (GR). The main role of GR is keeping reduced sulfhydryl groups of proteins with the participation of glutathione transhydrogenase. GR also participates in the removal of noxious substances from cells (xenobiotics and their metabolites, products of lipid peroxidation, etc.) through enzymatic reactions or reactions catalysed by glutathione S-transferase [4]. Also worthy of note is ceruloplasmin (CP) — a copper-binding protein. CP contributes to decreasing copper concentration in serum and prevents the participation of copper in free-radical reactions [12].

Inflammatory bowel disease constitutes a group of diseases of barely known aetiology. One of the possible causes may be free radical reactions increasing oxidative stress. Thus, the authors try to describe the antioxidant abilities of the organism in the course of the aforementioned diseases.

This study aims to assess the antioxidative barrier of the organism in patients with inflammatory bowel disease.

## Material and methods

### Material

The study group consisted of 80 patients at the Clinic of Jan Biziel University Hospital in Bydgoszcz, Poland. The 80 patients had IBD (mean age  $32.66 \pm 17.4$  years).

The reference group consisted of 19 healthy people (mean age  $22.24 \pm 3.64$  years), with no health conditions (fever, contagious diseases, blood clots, pregnancy, kidney and liver conditions, cancer, inflammations and hypertension were excluded as a contraindication during a general medical examination). Every person fasted till the last blood sample collection.

The study was conducted under the Declaration of Helsinki and the guidelines for Good Clinical Practice (GCP). Freely given written informed consent was obtained from every participant before the study. Approval was granted by Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University, Torun, Poland, ethics review board KB 551/2013.

### Methods

The material for biochemical analysis was venous blood collected in an amount of approx. 8 ml from the antecubital vein into lithium heparin tubes and tubes without anticoagulant. The blood samples were collected at 8.00 a.m. Then, the collected material was transported to the Department of Biochemistry of Nicolaus Copernicus University Collegium Medicum in Bydgoszcz. Tests were carried out on the same day, within approximately 1 hour of material collection. In the experiment, the activity of the following biochemical markers of oxidative homeostasis was assessed: oxidase ceruloplasmin (CP), glutathione (GSH), glutathione reductase (GR), malondialdehyde (MDA), glutathione peroxidase (GPX), glutathione S-transferase (GST) and superoxide dismutase (SOD-1).

It was assayed using an impeller-based method. From the blood drawn into tubes without anticoagulant (approximately 3 ml), serum was obtained by centrifugation of the material over 5 minutes at  $5000 \times g$ , then it was transferred to Eppendorf tubes and frozen at  $-80^{\circ}\text{C}$ . The prepared serum was stored to determine the activity of the oxidase ceruloplasmin (Cp).

Before preparing the hemolysate,  $500\mu\text{l}$  blood was collected to determine the levels of GSH in the erythrocytes. The remaining aliquot of blood (approx. 5ml) was centrifuged to obtain plasma, wherein the concentration of nitrate/nitrite ( $\text{NO}_2^-/\text{NO}_3^-$ ) was determined. The remaining cells were used for the preparation of the hemolysate, wherein the dialdehyde malonic concentration (MDA) and the activity of the enzymes: glutathione peroxidase (GPX), glutathione S-transferase (GST), glutathione reductase (GR) and superoxide dismutase (SOD-1) was determined.

The concentration of GSH was assayed using the Beutler method [13]. The principle of this method is based on the reaction of reduction of the disulfide compound — dithio-bis-2-nitrobenzoic acid (DTNB) by compounds containing sulfhydryl groups. In blood, free sulfhydryl groups unrelated to proteins are derived almost

entirely from GSH. The product of the described reaction is a compound of yellow colour. Colour density was measured at wavelength 412 nm. In the calculations, the molar absorption coefficient was used, which, when attached to the mentioned wavelength, is equal to  $13.6 \text{ [mmol}^{-1} \times \text{l} \times \text{cm}^{-1}]$ . The results were expressed in mmol/LRBC. The coefficient of variation for this method was 2.4%.

The activity of GR in erythrocytes was assayed by spectrophotometric measurement of NADP formation rate. NADP is the result of the reduction of glutathione oxidase in a reaction catalysed by glutathione [14]. Change of absorbance was measured at wavelength 340 nm, and the result was expressed in U/g Hb. The variation coefficient for this method was 3.8%.

The activity of GPX in erythrocytes was assayed by a two-stage Paglia and Valentine method [15]. In the first stage, GPX reacts with tert-butyl peroxide and reduced glutathione (GSH). The product of this reaction is glutathione disulfide (GSSG). The second stage involves the action of glutathione reductase (GR) reducing GSSG to GSH with the participation of NADPH + H<sup>+</sup> as a regulator. NADPH oxidation results in a reduction in absorbance at a wavelength of 340 nm, which is measured spectrophotometrically. The activity of GPX was calculated based on the loss of the reduced form of coenzyme in time (Wartburg test). In the calculations, the millimolar absorption coefficient for NADPH at wavelength 340 nm, equal to  $6.22 \text{ [mmol}^{-1} \times \text{l} \times \text{cm}^{-1}]$  was used. The results were expressed in U/g Hb, where  $1 \mu\text{mola}$  oxidation of NADPH in one minute at T = 25°C was adopted as a unit of enzyme activity. The coefficient of variation for this method was 2.9%.

Determination of GST activity in erythrocytes was performed according to the Habig method [15]. In this method, there is a decrease in absorbance (which is measured at a wavelength of 340 nm) due to the formation of a conjugate of glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB). The decrease in absorbance is proportional to the glutathione S-transferase activity. GST activity assay was carried out in the presence of phosphate buffer and CDNB. The results were expressed in nmol/CDNB-GSH/mg Hb/min.

SOD-1 activity in erythrocytes was determined using the Misra and Fridovich method, which is based on the inhibition of adrenaline oxidation reaction by superoxide dismutase at pH 10.2 [8]. The increase in absorbance was measured at a wavelength of 480 nm. It is proportional to the increase in the concentration of oxidation products of adrenaline. The activity of SOD-1 was expressed in U/g Hb. The amount of enzyme which inhibits the oxidation of adrenaline by 50% was adopted as a U unit. The coefficient of variation for this method was 6.3%.

The concentration of MDA in the erythrocytes was determined by the Placer et al. method, which is based on the reaction of a thiobarbituric acid and cer-

tain products of lipid peroxidation, mainly MDA, in an acidic environment and at elevated temperature [16]. This reaction produces a coloured product, the colour intensity of which was measured at a wavelength of 532 nm. In the calculations, the millimolar absorption coefficient of  $156 \text{ [mmol}^{-1} \times \text{l} \times \text{cm}^{-1}]$  was used. The result was expressed in mmol/g Hb. The coefficient of variation for this method was 3.5%.

The concentration of nitric oxide was determined using the indirect method according to Marlett, determining the concentration of nitrate/nitrite in plasma. The method is based on the reaction between the nitrate anion and the anion from N- (1-naphthyl) ethylenediamine, in a sulfanilic acid environment (Griess reaction) [17]. This reaction produces a coloured complex whose absorbance is measured at a wavelength of 545 nm. It is directly proportional to the concentration of nitrates and nitrites in the studied sample. The result was expressed in  $\mu\text{mol/L}$ .

Ceruloplasmin oxidase activity was determined using the method of Ravin [18]. The principle of the method is based on the oxidation of substrate p-phenylenediamine (PPD) by ceruloplasmin with a final purple-coloured product. Absorbance measurement was made at a wavelength of 530 nm. This product is the so-called 'principle of Bandrowski' (a product formed from three molecules of the substrate). Results were expressed in International Units.

### Statistical analysis

Statistical analysis was made using the software SPSS21. Where available, the measured data were described as mean with standard deviation (SD) or median with minima and maximal values. The normality of distribution was checked using the Shapiro-Wilk test. According to need, for data sets with normal distribution, the t-test was applied, and for the other data sets, the U-Mann Whitney test was applied. P-value was set at 0.05. The relationship between the studied parameters and the age of the patients was used to calculate the Pearson correlation. The ROC analyses were used to evaluate the values of the studied parameters in both groups of patients.

## Results

There were statistically significant differences in oxidative stress parameters observed between the study group and the control group (Tab. 1).

The mean concentration of the reduced glutathione in erythrocytes was higher in the study group compared with the control group ( $p \leq 0.01$ ). The activity of glutathione peroxidase was lower in the study group compared with the control group ( $p \leq 0.01$ ). The activity

**Table 1.** Differences in oxidative stress parameters observed between the study group and control group

| Parameter   | Study group<br>(n = 80) |          | Reference group<br>(n = 19) |          | p-value |
|---|-------------------------|----------|-----------------------------|----------|---------|
|   | Mean                    | SD       | Mean                        | SD       |         |
| HT  | 40.15                   | 4.206    | 43.34                       | 3.253    | 0.003   |
| GSH (mmol)  | 2.793                   | 0.3730   | 2.208                       | 0.1625   | ≤ 0.01  |
| GPXp (U)  | 184.480                 | 243.9192 | 246.047                     | 48.2888  | n.s.    |
| GPX <sub>RBC</sub> (U)  | 15.336                  | 2.2086   | 18.911                      | 2.2573   | ≤ 0.01  |
| GST (nmol)  | 2.872                   | 0.6929   | 2.495                       | 0.4236   | 0.025   |
| GR (U)  | 56.278                  | 9.4105   | 55.147                      | 11.2139  | n.s.    |
| SOD-1 (U)   | 2471.19                 | 148.555  | 2805.26                     | 184.771  | ≤ 0.01  |
| MDA (mmol)  | 0.28362                 | 0,024803 | 0.25289                     | 0.033637 | ≤ 0.01  |
| NO <sub>2</sub> <sup>-</sup> /NO <sub>3</sub> <sup>-</sup> (μmol/L) | 1.4064                  | 1.24503  | 0.8121                      | 0.63482  | 0.047   |
| CP (IU)   | 1206.121                | 415.9030 | 1340.358                    | 542.4489 | n.s.    |

CP — oxidase ceruloplasmin, GPX — glutathione peroxidase, GPXp — glutathione peroxidase (plasma), GPX<sub>RBC</sub> — glutathione peroxidase (red blood cells), GR — glutathione reductase, GSH — glutathione, GST — glutathione S-transferase, HT — haematocrit, MDA — malondialdehyde, NO<sub>2</sub><sup>-</sup> — nitrate, NO<sub>3</sub><sup>-</sup> — nitrite, n.s. — not significant, O<sub>2</sub><sup>•-</sup> — singlet oxygen, O<sub>2</sub><sup>-</sup> — superoxide anion, O<sub>3</sub> — ozone, SD — standard deviation, SOD-1 — superoxide dismutase

of this enzyme in erythrocytes was higher in the study group compared with the control group ( $p \leq 0.01$ ). There was also higher activity of the glutathione-S-transferase observed in the study group compared with the control group ( $p = 0.025$ ). The activity of SOD-1 was significantly lower in the study group compared with the control group ( $p \leq 0.01$ ). The concentration of MDA was significantly higher in the study group compared with the control group ( $p \leq 0.01$ ). The concentration of nitric oxide in plasma (measured indirectly based on the concentration of nitrates/nitrites) was significantly higher in the study group compared with the control group ( $p = 0.047$ ). Values of Ht were significantly lower in the study group compared with the control group ( $p = 0.003$ ). Other statistically significant differences between the study and the control group were not observed.

The correlation between the values of the studied parameters and the age of the patients was analysed by calculating Pearson's correlations. There was no statistically significant correlation between these parameters with the age of the patients. These data are presented in Table 2.

Analysing the influence of sex on the values of the parameters studied in the group of patients, a statistically significantly higher value of haematocrit was found in the group of men than in women ( $43.47 \pm 3.15$  vs.  $38.06 \pm 3.37$ ;  $p \leq 0.01$ ) and a significantly lower value of GSH and CP in the group of men than women (respectively:  $2.65 \pm 0.31$  vs.  $2.89 \pm 0.38$ ;  $p \leq 0.05$  and  $949.1 \pm 213.7$  vs.  $1368.73 \pm 431.9$ ;  $p \leq 0.01$ ). For the remaining parameters, no statistically significant differences were found between the groups of men and women. The data is presented in Table 3.

In the study group compared with the control group, no changes in the levels/concentration of the measured parameters of oxidative stress were observed in GPX<sub>p</sub>, GR and CP. On the contrary, it was shown that in the study group compared with the control group, there was a significant decrease of about 23.31 and 13.52% in the level/concentration of GPX<sub>RBC</sub> and SOD respectively. The study group demonstrated significant increases of 20.95%,

**Table 2.** Correlation of the values of the assessed parameters with the age of the patients

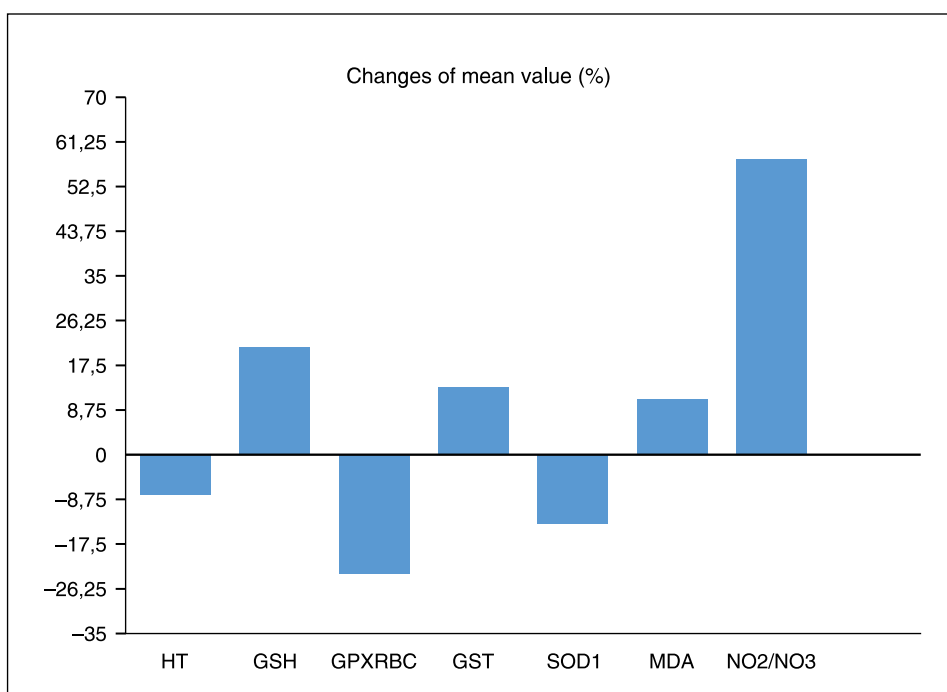
| Parameter   | Age     |         |
|---|---------|---------|
|   | r-value | p-value |
| HT  | 0.117   | 0.517   |
| GSH (mmol)  | -0.316  | 0.073   |
| GPXp (U)  | 0.274   | 0.122   |
| GPX <sub>RBC</sub> (U)  | 0.178   | 0.240   |
| GST (nmol)  | 0.294   | 0.097   |
| GR (U)  | 0.014   | 0.940   |
| SOD-1 (U)   | -0.252  | 0.157   |
| MDA (mmol)  | -0.158  | 0.379   |
| NO <sub>2</sub> <sup>-</sup> /NO <sub>3</sub> <sup>-</sup> (μmol/L) | -0.034  | 0.852   |
| CP (IU)   | -0.149  | 0.406   |

CP — oxidase ceruloplasmin, GPX — glutathione peroxidase, GPXp — glutathione peroxidase (plasma), GPX<sub>RBC</sub> — glutathione peroxidase (red blood cells), GR — glutathione reductase, GSH — glutathione, GST — glutathione S-transferase, HT — haematocrit, MDA — malondialdehyde, NO<sub>2</sub><sup>-</sup> — nitrate, NO<sub>3</sub><sup>-</sup> — nitrite, O<sub>2</sub><sup>•-</sup> — singlet oxygen, O<sub>2</sub><sup>-</sup> — superoxide anion, O<sub>3</sub> — ozone, SOD-1 — superoxide dismutase

**Table 3.** Comparison of the values of the studied parameters, broken down by gender

| Parameter  | Women<br>n = 49 |        | Men<br>n = 31 |        | p-value |
|--|-----------------|--------|---------------|--------|---------|
|  | Mean            | SD     | Mean          | SD     |         |
| HT   | 38.06           | 3.37   | 43.61         | 3.15   | ≤ 0.01  |
| GSH (mmol)   | 2.88            | 0.38   | 2.65          | 0.031  | ≤ 0.01  |
| GPXp (U)   | 151.38          | 38.12  | 236           | 387    | 0.128   |
| GPX <sub>RBC</sub> (U)                                 | 15.39           | 2.22   | 15.25         | 2.22   | 0.785   |
| GST (nmol)   | 2.85            | 0.69   | 2.9           | 0.71   | 0.750   |
| GR (U)   | 57.08           | 9.6    | 55.01         | 9.12   | 0.343   |
| SOD-1 (U)  | 2471.43         | 141.01 | 2470.81       | 162.05 | 0.986   |
| MDA (mmol)   | 0.28            | 0.02   | 0.29          | 0.03   | 0.370   |
| NO <sub>2</sub> /NO <sub>3</sub> <sup>-</sup> (μmol/L) | 1.3             | 0.81   | 1.57          | 1.73   | 0.31    |
| CP (IU)  | 1368.73         | 431.85 | 949.1         | 213.68 | ≤ 0.01  |

CP — oxidase ceruloplasmin, GPX — glutathione peroxidase, GPXp — glutathione peroxidase (plasma), GPX<sub>RBC</sub> — glutathione peroxidase (red blood cells), GR — glutathione reductase, GSH — glutathione, GST — glutathione S-transferase, HT — haematocrit, MDA — malondialdehyde, NO<sub>2</sub> — nitrate, NO<sub>3</sub> — nitrite, O<sub>2</sub> — singlet oxygen, O<sub>2</sub><sup>-</sup> — superoxide anion, O<sub>3</sub> — ozone, SD — standard deviation, SOD-1 — superoxide dismutase

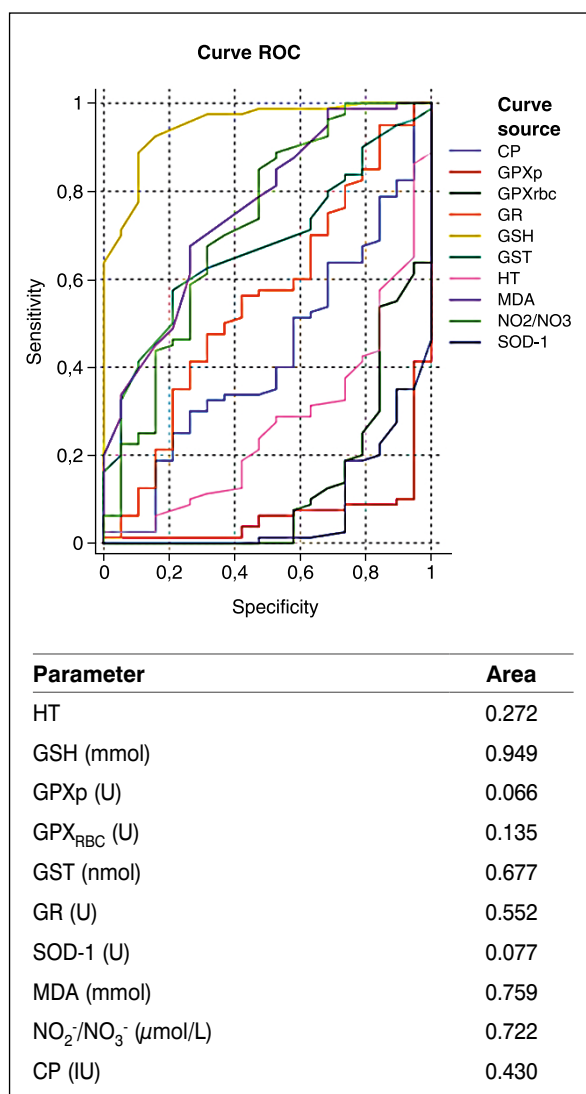


**Figure 1.** Percentage of changes in mean values of selected measured parameters of oxidative stress observed between study group and control group

13.13%, 10.83% and 57.74% in the level/concentration of GSH, GST, MDA and NO<sub>2</sub>//NO<sub>3</sub><sup>-</sup> respectively (Fig. 1).

To assess the values of the tested parameters in both groups, the ROC analysis was also performed — assessing the diagnostic power of the parameters

tested. The obtained results of the ROC analysis, ROC curves and the values of the area under the curves, are presented in Figure 2. The greatest diagnostic power differentiating between the groups is in GSH, MDA, NO<sub>2</sub>/NO<sub>3</sub>, GST, GPX, and SOD.



**Figure 2.** ROC analysis assessing the differentiating value of the examined parameters between the study group and the control group

## Discussion

The development of diseases based on free radicals may take place when the intensity of the oxidative stress exceeds the adaptability of the organism. Prevention of results of the extensive ROS production elements of the antioxidative barrier of the organism should be achieved by an integrated and efficient system allowing control of free radical production and defending against their biological effects. Such a system consists of both enzymatic and nonenzymatic mechanisms making ROS harmless.

IBD constitutes a group of diseases characterized by various and not fully known aetiology. One of the possible causes of their occurrence may be free radical reactions and a disorder of balance between levels of

prooxidants and antioxidants. Drugs used to treat the aforementioned diseases show both anti-inflammatory and antioxidant action, which is important taking into consideration the free radical causes of IBD [19]. The assumption that increased activity of antioxidative compounds may constitute a defence against the influence of oxidative stress may be true. Their decreased activity may participate in lowering an organism's abilities to defend against oxidative stress and cause the development of free radical diseases.

The main limitation of the previous studies is the relatively low number of coherent results explaining associated problems comprehensively. The present study constitutes another step toward better understanding free radical and antioxidative processes in patients with IBD. Previous studies on oxidative stress concerned mostly single parameters, therefore a study was undertaken in which many of them were analysed simultaneously.

ROS are produced in many biological processes. Released in physiological amounts, they play the role of mediators and regulators providing for the normal functioning of cells [20]. ROS influence on cells depends on their concentration and duration of action, and their production should be under strict control of both the enzymatic antioxidant system and the nonenzymatic antioxidant system. When such a defence system is inefficient and the production of ROS increases, there may be a disturbance in the balance between prooxidant and antioxidant processes within the cell. This phenomenon is called oxidative stress. Its consequences are damage to cell elements or even disorders of cells and tissue function [21]. The mechanism of ROS production is cascading. During the first stage of the reaction, an electron is joined to the oxygen producing a superoxide radical anion [22]. The superoxide radical anion is a basis for other ROSs. SOD participates in the decomposition of the superoxide radical anion into oxygen and hydrogen peroxide. Vaiopoulou et al. [23] showed decreased activity of this enzyme in neutrophils from tissue cultures in patients with IBD compared to healthy people. Rana et al. [18] showed higher activity of this enzyme in the erythrocytes of patients with UC compared with the reference group. The authors' studies showed a decrease in the activity of SOD-1 in erythrocytes of patients with IBD compared with the control group. Decreased activity of SOD-1 may participate in the accumulation of the superoxide radical anion. Its high level may also inhibit the activity of GPX. The authors' studies confirm a decrease of GPX activity in erythrocytes of patients with IBD compared to the control group. Similar outcomes were observed by Krzystek-Korpacka et al. [24] There was observed decreased activity of this enzyme in erythrocytes of people with IBD treated for anaemia. On the other

hand, Akman et al. [25] showed a lack of statistically relevant differences in GPX activity in the serum of patients with IBD compared to healthy people. The results of the authors' study were similar: statistically relevant differences in GPX activity in the serum of patients with IBD compared to healthy people were not observed. Anaemia belongs to systemic complications of IBD. It constitutes a cause of quality of life decrease and hospitalization [7]. Haematological diagnostics of IBD consist of measurements of haemoglobin (Hb) concentration, red blood cell count, white blood cell count, haematocrit (Ht) and erythrocyte sedimentation rate. The present study results for Ht were significantly lower in the study group compared to the reference group. GSH is the main element of defence against oxidative stress. Ruan et al. [10] observed decreased GSH levels in patients with IBD compared to a control group. Rana et al. [18] showed a decreased level of GSH in patients with CU compared with the reference group. Ravin et al. [26] did not observe statistically relevant differences in GR levels in tissues of patients with chronic CU and CD, but they observed that GSH concentration is increased during the remission of CU compared to the active period of the disease. The study results showed an increase in the GSH level in IBD patients compared with the control group. Glutathione is one of the most important elements of the antioxidative cell defence system due to its ability to reduce peroxides and keep normal levels of –SH groups within proteins [27]. Moreover, it plays the role of an intracellular high-capacity redox buffer and ROS destroyer. GST activity also helps to assess disorders of the antioxidant barrier in erythrocytes. The biological action of this enzyme is based on its participation in the second phase of the detoxication of xenobiotics. GST may also participate in the regulation of GPX activity [28]. The authors' studies showed increased activity of this enzyme in the study group compared to the control group. GR plays an adjuvant function supporting the main elements of the antioxidative protective barrier. It reduces oxidative glutathione and brings back its antioxidative abilities, which allows for its reuse in reactions catalysed by GPX and GST [29]. The authors' studies showed a lack of statistically relevant differences in GR activity between the study group and the reference group. Results of damage caused by oxidative stress can be measured indirectly by the concentration of thiobarbituric acid [30] — MDA is the most used. MDA is a result of lipid peroxidation and its concentration increases in the case of increased ROS production in the body. It causes a change in the membrane cell permeability, disturbances of oxidative phosphorylation within mitochondria, and as a consequence – induction of apoptosis [28]. Statistically relevant increase in MDA concentration constitutes a useful

indicator of disorders of the antioxidative barrier of the body. Alzoghaibi et al. [31] showed a statistically relevant increase in MDA concentration in the serum of patients with Crohn's disease compared with the reference group. Despite MDA concentration in patients with CU being also significantly higher than in healthy people, the aforementioned data were not statistically relevant and further research is needed. The authors' studies showed increased MDA concentration in the study group compared with the control group. On the other hand, Trougakos et al. [32] showed a lack of differences in MDA concentration between IBD patients and healthy people. Nitric oxide (NO) is also a nonenzymatic antioxidative factor. It plays a significant role in the regulation of the cell membrane tension, ROS capturing and protection against lipid peroxidation [33]. The role of NO is twofold: it is an antioxidant and it participates in ROS production [34]. The authors' study consisted of measuring the nitrites to nitrates ratio, which allows for assessment of the NO metabolism. A statistically significant increase of the NO metabolism in the blood was observed in the study group compared to the reference group. Similar results were shown by Avdagic et al. [35]: an increase in NO level was observed in patients with IBD compared with the control group. The increase in the concentration of NO in patients with IBD is a result of increased activity of NO synthesis in this group of diseases [1]. The ability to defend against ROS also depends on the action of endogenous proteins which have antioxidant properties. CP belongs to such proteins – it binds transition metal ions, which decreases free radical reactions [12]. No statistically relevant differences concerning the concentration of this protein between the study group and the reference group were observed. Tüzün et al. [36] showed that overexpression of CP might be engaged in the onset and development of Crohn's disease.

## Conclusions

Prooxidative factors play an essential role in the pathogenesis of IBD.

Due to the still unknown etiopathology of IBD, research on imbalances between pro-oxidants and antioxidants should be continued in larger groups of patients.

The undertaken research is of a pilot nature and was conducted with the use of manual methods of determination. Subsequent research should use automated methods for their standardization and possible use on a large scale in everyday clinical practice.

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