Evaluation of antioxidant activity in the erythrocytes in patients with Down syndrome

Ocena aktywności antyoksydacyjnej w erytocytach u pacjentów z zespołem Downa

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STRESZCZENIE

Celem pracy byla ocena aktywności enzymów antyoksydacyjnch: dysmutazy ponadtlenkowej (SOD), peroksydazy glutationowej (GSH-Px), reduktazy glutationowej (GSSG-R) oraz stężenia dialdehydu malonowego (MDA) w erytrocytach osób z zespołem Downa (ZD). **Materiał i metody.** Badaniem objęto grupę 66 pacjentów (24 dzieci i 42 dorosłych)z trisomią 21 pary chromosomów. Grupę kontrolną stanowiło 59 osób zdrowych (24 dzieci i 35 dorosłych). Aktywność SOD, GSH-Px, GSSG-R oraz stężenie MDA w erytrocytach oznaczono metodami spektrofotometrycznymi. **Wyniki.** Wykazano wyższą aktywność enzymów antyoksydacyjnych: SOD, GSH-Px, GSSG-R u osób z ZD w porównaniu do grupy kontrolnej, która malała wraz z wiekiem. Stwierdzono podwyższone stężenie MDA u osób z ZD, narastające wraz z wiekiem. Uzyskane wyniki wskazują na nasilenie procesów lipidowej peroksydacji w erytrocytach osób z ZD.

Słowa kluczowe: enzymy antyoksydacyjne, peroksydacja lipidów, zespół Downa

ABSTRACT

The aim of this study was to evaluate the activity of antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSSG-R) and serum malondialdehyde (MDA) levels in the erythrocytes in patients with Down syndrome (DS). **Materials and methods.** The study included 66 patients (24 children and 42 adults) with Trisomy 21. The control group consisted of 59 healthy individuals (24 children and 35 adults). The activity of SOD, GSH-Px, GSSG-R and MDA levels in erythrocytes were determined spectrophotometrically. **Results.** We found a greater activity, which decreased with age, of antioxidant enzymes: SOD, GSH-Px, GSSG-R in patients with DS compared with the control group. MDA levels, which increase with age, were elevated in patients with DS. The results indicate an intensification of the lipid peroxidation processes in the erythrocytes of patients with DS.

Keywords: antioxidant enzymes, lipid peroxidation, Down syndrome

INTRODUCTION

The oxidation and anti-oxidation balance is one of the elements of system homeostasis. Under physiological conditions, reactive oxygen species (ROS) are formed. An uncontrolled increase in ROS concentration leads to a chain of radical reactions resulting in the degradation of proteins, lipids, sugars and nucleic acids [1,2].

A physiological imbalance between ROS generation and inactivation leads to oxidative stress which is a series of biochemical reactions that may result in cell damage and death. The participation of oxidative stress has been proven in the pathogenesis of many acute and chronic diseases, including those of the nervous system [3]. There is information available that oxidative stress plays a role in the pathogenesis of Down syndrome [1].

The pathological implications of ROS reactions and oxidative stress leading to the increased concentrations of lipid peroxidation products and changes in the activity of enzymes that protect against ROS and low-molecular-mass antioxidant concentrations arouse great interest [1].

The studied biological free-radical process is lipid peroxidation in which the oxidation and degradation of polyunsaturated fatty acids contained in the membrane lipids take place. Lipid peroxidation - a direct manifestation of adverse changes induced by ROS activity - is an avalanche process. It provides a continuous supply of free radicals that initiate further peroxidation reactions.

Peroxides formed in the course of this process are further metabolized reaching stable non-radical compounds containing: aldehyde, ketone, hydroxyl, carboxyl, peroxyl and epoxy functional groups [4]. Malondialdehyde (MDA) is considered a marker of the lipid peroxidation process [1].

The basic endogenous enzymes involved in ROS removal are superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and glutathione reductase (GSSG-R). Changes in the activity of these enzymes reflect the progress of the occurring lipid peroxidation processes. Vitamins C and E also possess the antioxidant properties [5].

The primary criterion for assessing the presence of free radicals is to detect the physiological protective effect of

the antioxidant system. An estimation of the changes taking place in this system by measuring the activity of SOD, GPx, GR and MDA levels enables assessing the increases or decreases of ROS formation and other free radicals [6].

Erythrocytes are a valuable diagnostic material. We decided to determine the antioxidant activity in red blood cells because they are susceptible to oxidative damage due to the high content of polyunsaturated fatty acids in their cell membranes [7]. Tan and Yeung [8] believe that a high concentration of oxygen and hemoglobin in erythrocytes makes them potentially powerful promoters of the oxidative processes. Blood cells are rich in antioxidant enzymes; they may reflect any changes in the catalytic activity of these enzymes in remote less accessible tissue [9].

Several studies conducted *in vivo* and *in vitro* indicate a link between the effects caused by compounds that generate ROS and the level of antioxidant enzymes (SOD, GPx, GR) and lipid peroxidation products, including MDA [2.10].

We demonstrated disorders in this area in experimental seizures and epilepsy [5:12-15], in migraine headaches [16,17] and also in the serum in children with Down syndrome [11].

AIM

The aim of this study was to evaluate the activity of antioxidant enzymes: SOD, GSH-Px, GSSG-R and serum MDA levels in the erythrocytes in patients with DS. The correlation between the activity of the tested compounds and the age of the patients was assessed.

MATERIALS AND METHODS

The study included 66 patients with DS (24 children and 42 adults) aged from 0.8 months to 53 years. The control group consisted of 59 healthy individuals (24 children and 35 adults) aged 1.5 to 65 years, with Down syndrome and organic brain damage ruled out.

The material for the study was obtained simultaneously with blood collection for routine basic examinations.

The activity of antioxidant enzymes (SOD, GSH-Px, GSSG-R) and MDA levels in erythrocytes were determined spectrophotometrically using the EPOLL-20 photometer. To assess the activity of SOD, the method according to Sykes et al. was used [18], GSH-Px - according to Paglia and Valentine [19], GSSG-R- according to Mize and Longdan [20]. MDA levels were determined using the method developed by Buege and Aust [21].

The obtained results are presented together (children and adults) in the form of regression curves.

Statistical analysis of the results was performed using the Statistica software. The study was approved by the Bioethics Committee of the Medical University of Bialystok.

RESULTS

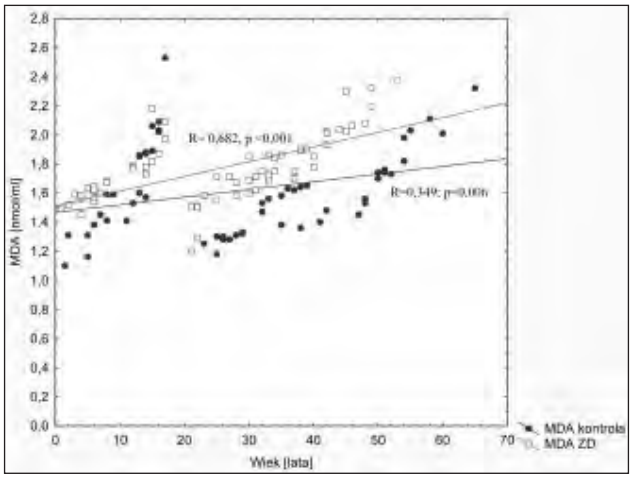

Fig. 1. MDA concentration in erythrocytes in people with Down syndrome and in controls

Statistically significant higher accumulation of this compound was found in patients with DS compared with the control and an increase of its concentration with age.

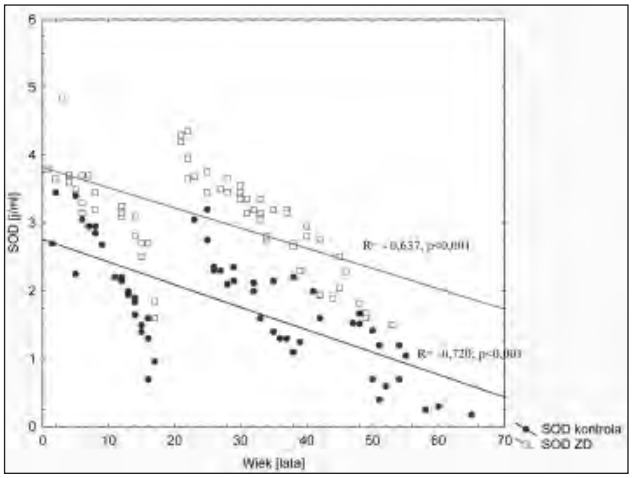

Fig. 2. Activity of SOD in erythrocytes in patients with Down syndrome and in controls

A statistically significant increase in the activity of this enzyme, which decreased with age, was found in patient with DS.

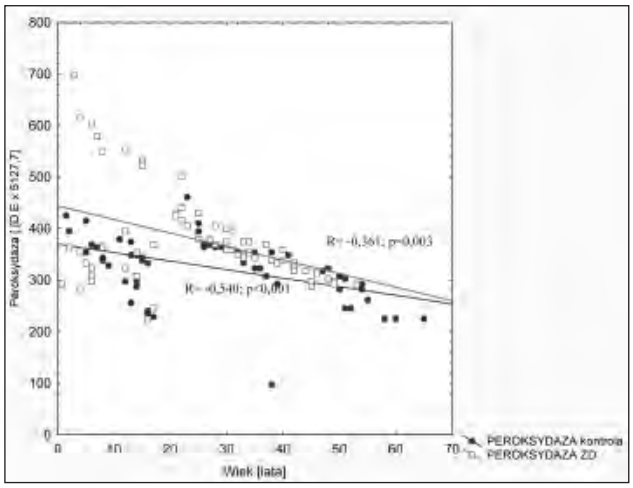

Fig. 3. Activity of GSH-Px in erythrocytes in people with Down syndrome and in controls

A significant increase in the activity of this enzyme, which decreased with age, was found in patients with DS compared with the control.

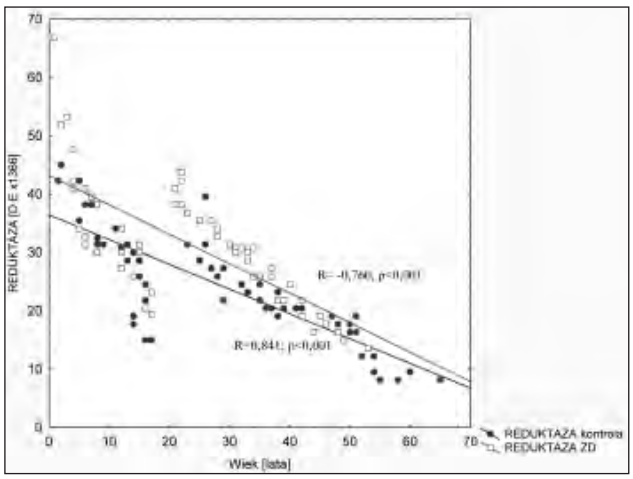

Fig. 4. Activity of GSSG-R in erythrocytes in patients with Down syndrome and in controls

And here it was also higher in patients with DS in comparison with the control group, and decreased with age.

DISCUSSION

The obtained results show that the oxidation of lipids to peroxide form is more severe in people with Down syndrome as compared with the group of healthy subjects. Higher MDA levels, an indicator of the dynamics of these processes and the degree of destruction of membranes by oxygen free radicals, were observed in the erythrocytes in children and adults.

The result of an increase in lipid peroxidation processes is most often secondary activation of antioxidant enzymes. These enzymes are a basic endogenous mechanism that protects against the effects of oxygen free radicals. SOD is involved in the direct elimination of superoxide anion, catalyzing its decomposition into molecular oxygen and hydrogen peroxide [2]. In our study we found a significant increase in SOD activity in the erythrocytes in patients with DS compared with the control group. In the group of children, these differences were not as great as in the adults, in whom the activity of SOD in the studied group increased by 46.93%. The obtained results are consistent with the analysis conducted by Thiel [22]. The author believes that an excess of genetic material increases the expression of this enzyme resulting in the overproduction of the RNA template for this protein [22]. Our results indicate that the activity of this enzyme decreases significantly with these patients' age.

GSH-Px catalyzes the oxidation of reduced glutathione. In oxidized form, glutathione may reduce lipid peroxides and hydrogen peroxide. It is believed that GSH-Px is of the utmost importance in protecting brain tissue against oxidative damage [5].

In our study we found a significant increase in GSH-Px activity in the erythrocytes in patients with DS compared with the control group. This indicates the activation of antioxidant defense associated with the need to eliminate excess hydrogen peroxide. The activity of this enzyme in adult patients with DS was higher by over 12% compared to the values obtained in the control. However, it is important that the activity of this enzyme shows a downward trend compared with children with DS.

Glutathione reductase (GSSG-R) plays a major role in the metabolism of glutathione. In our study GSSG-R activity in erythrocytes is as follows: in the group of people with DS, its activity is higher compared with the control group. Simultaneously, we found decreased activity of this enzyme depending on the studied patients' age.

Muchova et al. presented different results. [23]. They reported significantly decreased GSH-Px in individuals with DS. Also the authors found no differences in GSSG-R levels compared with the controls.

Pastor et al. [24], evaluating people with DS aged 1 to 50 years, reported significant changes in the activity of antioxidant enzymes in the erythrocytes in patients with DS compared with the control group. SOD activity increased by 37%, GSH-Px by 8.4%, GSSG-R by 37.4%. Analyzing the obtained results, the authors found a significant decrease with age in the activity of SOD and GSSG-R, whereas GSH-Px activity was higher in older patients. At the same time, they did not observe differences in the values of α-tocopherol levels between the patients with Down syndrome and the control group [24].

Our results are consistent with those obtained by Ordonez et al. [7]. The researchers obtained a statistically higher activity of SOD, GSH-Px, catalase (CAT) and glucose-6-phosphate dehydrogenase (G6PDH) in the erythrocytes in patients with DS. The increase in enzyme activity was 35.2%, 15.3%, 6.0%, 14.9%, respectively.

The processes of lipid peroxidation, particularly in the CNS, have been studied in our Department for many years. In examining the imbalance of oxidants-antioxidants in the serum of 41 children with DS (2007), we obtained an increase in MDA levels compared to the control, but the differences were not statistically significant. The activity of SOD and GSSG-R showed no significant differences compared with the control group. Only the activity

of GSH-Px was significantly higher in children with DS [11]. In contrast, in studies also conducted in our Department on antioxidant activity in the serum and saliva of patients treated for epilepsy, completely different results were obtained [12]. The activity of the studied enzymes (SOD, GSH-Px, GSSG-R) was significantly lower than in the control group.

Śmigielska et al. [11] observed no significant differences in GSH-Px activity in the serum in patients with DS depending on the gender of the respondents. Similar results were obtained by Habif et al. [25] and Andersen et al. [26], in contrast to the French researchers [27] according to whom the level of the GSH-Px enzyme is higher in the female population.

The results presented in this paper are partly consistent with the research conducted by Gerli et al. [28]. The authors, analyzing antioxidative activity in the erythrocytes of adults with DS, found a statistically significant increase in the activity of SOD and GSH-Px. Only the activity of GSSG-R and catalase remained normal.

According to Garcez et al. [29], the activity of SOD and catalase in the serum of patients with DS is significantly higher compared with healthy individuals.

Percy et al. [30], assessing the activity of antioxidants in the erythrocytes of patients with DS and Alzheimer's disease, obtained similar results to ours, although the activity of GSSG-R showed no changes dependent on the manifestation of Alzheimer's disease. These authors also suggest that the antioxidant activity disorders are responsible for the early manifestation of the disease [30].

Research by Pastore at al. from 2003 showed a decrease in the levels of all forms of glutathione in children with DS, which indicates a decrease of activity of this enzyme [31].

When studying the activity of SOD and GSH-Px in the erythrocytes in patients with DS, Muchova et al. [32] also obtained similar results to ours. The activity of SOD and GSH-Px was significantly higher compared with the control who were the healthy siblings of children with DS. No significant differences were found in the activity of GSSG-R and CAT.

Meguid Nagwa Abdel et al. [33], analyzing the activity of SOD and GSH-Px in the erythrocytes of Egyptian children, obtained the following results: the activity of SOD and GSH-Px indirectly affects the amount of the gene. According to the researchers, in a population of individuals with full trisomy and translocation, the activity of SOD and GSH-Px is higher; while in patients with a mosaic, the activity of the enzymes remains normal.

Studies of the cerebral cortex of fetuses with DS [34] showed an increase of SOD activity by 60 ± 5% compared with the control group of fetuses at similar gestational age. In addition, the authors assessed the formation of MDA in homogenates of the cerebral cortex of fetuses with DS, which was about 36 ± 4% higher compared with the control group.

Other studies [35], conducted also post mortem, suggest that the neurons of patients with DS show a 3 to 4-fold increase in intracellular ROS and the lipid peroxidation level. The authors argue that the DS patients' neurons have defects in the metabolism of reactive oxygen species resulting in their accelerated apoptosis. This defect may contribute to the occurrence of mental retardation in the early period of life of patients with DS and their premature aging..

Carratelli et al. [36] determined the reactive oxygen species in the serum in children with Down syndrome. The authors found an increase in ROS levels in the studied material. According to their opinion, the accumulation of ROS results in the increased oxidative stress and is responsible for premature aging of individuals with DS.

De Haan and his team [37] showed higher SOD activity in the brain, lungs and thymus gland in fetuses with DS, and lower activity in the liver compared with the control group. GSH-Px activity was not changed in these structures, whereas in the liver was lower in patients with DS compared with the control.

The results of the study by Gromadzinska et al. [38] indicate a lower concentration of selenium in whole blood, RBC and plasma in patients with DS. Catalytic activity of GSH-Px in the erythrocytes in children with DS was significantly higher, while in the plasma significantly lower compared with a group of healthy children. According to the authors, the concentration of lipid peroxides, expressed as MDA levels is lower in patients with DS.

The opposite results were obtained by Slbodan V Jovanovic et al. [39]. The author, studying MDA levels in the urine samples of patients with DS, found a significant increase in MDA levels compared with the control [39].

Zitnanova et al. [40] did not find changes in the values of total antioxidant status (TAS) in the serum of children with Down syndrome.

On the basis of our own results and the literature data, it can be concluded that in patients with DS the oxidant and antioxidant balance is disturbed. The data obtained thus are far often contradictory, which proves the necessity to continue the research. Our results indicate a close link between ROS formation and the activity of antioxidant enzymes (SOD, GSH-Px, GSSG-R), serum lipid peroxidation products (MDA) and total antioxidant potential in the course of many diseases, including Down syndrome. Changes in the markers of lipid peroxidation processes can be a valuable source of information about the development and progression of the disease and facilitate the selection of the appropriate therapy.

CONCLUSIONS

Patients with Down syndrome had elevated MDA levels in erythrocytes compared with the control group. Simultaneously, we observed an increased accumulation with age which is regarded as a marker of increased oxidation of membrane lipids.

We found a significantly higher activity of antioxidant enzymes (SOD, GSH-Px, GSSG-R) in patients with Down syndrome compared with the control group. The activity of these enzymes decreases with age, which may cause accelerated aging.

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