Wspolczesna Onkol 2012; 16 (2): 165–169

DOI: 10.5114/wo.2012.28797

Original paper

Parameters of antioxidant barrier in different histopathologic types of pediatric cancers

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Aim of the study: The goal of this study was to evaluate the activities of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and the levels of glutathione (GSH) and ischemia-modified albumin (IMA), as potential markers in different histopathologic types of pediatric neoplasms. No studies on this subject have been reported to date.

Material and methods: SOD, GSH-Px, GSH, and IMA were measured before oncologic treatment in 129 children with neuroblastoma (NB), soft tissue sarcomas (STS), brain tumors, Hodgkin's disease (HD), and acute leukemias, and in 30 healthy controls.

Results: The statistical significance of SOD was observed in patients with brain tumors (median 1840.2 U/g Hb, $p = 0.0500$). The level of GSH was significantly higher in patients with NB (median 6.38 U/g Hb, $p = 0.0031$) and leukemias (5.16 U/g Hb, $p = 0.0200$). IMA was statistically significant in cases of STS, NB, and leukemias compared to healthy children ($p = 0.0244$, $p = 0.0069$, and $p = 0.0000$, respectively). The activity of GSH-Px was not statistically significant.

Conclusions: The antioxidant barrier in all types of pediatric cancers is disturbed. None of the measured parameters was specific enough to represent a reliable marker for any particular histopathologic type of children's neoplasm.

Key words: histology, superoxide dismutase, glutathione peroxidase and glutathione, IMA, pediatric cancer.

Introduction

The neoplastic process can be divided into three stages: initiation, promotion, and progression [1]. Reactive oxygen species (ROS) likely play a role in these steps by causing DNA mutations and subsequent damage during initiation. Additionally, they contribute to abnormal gene expression, blockage of cell-to-cell communication, and modification of second-messenger systems in the promotion stage. The net effect of these actions is an increase in cell proliferation or a decrease in apoptosis of the initiated cell population. Further DNA alterations to the initiated cell population occur in the progression stage [2, 3]. ROS may originate from both exogenous and endogenous sources. Exogenous sources include environmental agents, radiation, therapeutic agents, and tobacco smoke. Endogenous sources include mitochondria, peroxisomes, and inflammatory cell activation [2]. In physiological conditions, low levels of ROS play a protective role in the organism, while increased generation of free radicals is associated with tissue or DNA damage [4].

The balance between the production and removal of ROS is controlled by a variety of DNA repair enzymes and antioxidants [5]. The antioxidant system is comprised of low molecular weight antioxidant molecules, such as glutathione (GSH), and various enzymes, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). GSH participates in the process of scavenging reactive electrophiles and protects cells by reducing disulfide bonds of cytoplasmic proteins to cysteines [6]. The SOD, which catalyzes the formation of H_2O_2 from superoxide radicals, represents the first line of enzymatic defense against ROS. The GSH-Px reduces lipid or non-lipid hydroperoxides [7].

In recent years, ischemia modified albumin (IMA) has emerged as a new marker of ischemia and oxidative stress that originates as a consequence of tissue hypoxia [8]. The production of IMA may be correlated with the production of free radicals during ischemia and/or reperfusion, reduced oxygen tension, acidosis, and cellular alterations (e.g., disruption of the sodium and calcium pump) [9].

The aim of this study was to measure the activity of GSH-Px and SOD, and the levels of GSH and IMA in children with various cancers. We observed the behavior of these potential markers according to the histopathological characteristics of these tumors.

Material and methods

Patients

One hundred twenty-nine patients with soft tissue sarcomas (STS), neuroblastoma (NB), brain tumors, leukemia and Hodgkin's disease (HD) who were diagnosed and treated in the Department of Pediatrics, Hematology, Oncology, and Endocrinology, Medical University of Gdansk, Poland from 2006 to 2009 were enrolled in this study. In this study, there were 16 patients with STS

(8 females; 8 males) ranging in age from 1.51 to 18.01 years (mean 9.44 years; median 9.35 years). Another 16 patients had NB (5 females; 11 males) ranging in age from 0.12 to 15.55 years (mean 3.08 years; median 0.92 years). This study also included 20 patients with brain tumors (10 females; 10 males) ranging in age from 0.62 to 16.6 years (mean 10.7 years; median 12.1 years), 56 children with leukemia (27 females; 29 males) ranging in age from 0.85 to 18.0 years (mean 6.99 years; median 5.73 years), and 21 patients with HD (13 females; 8 males) ranging in age from 3.60 to 17.6 years (mean 13.9 years; median 15.2 years). The mean and median age of the control patients did not differ from any of the cancer patients ($p > 0.05$). All patients were examined before starting the first cycle of chemotherapy. The different histopathological types of cancers were established by open biopsy or the complete excision of the primary tumor. Pathological diagnoses were confirmed at two independent histopathological centers.

Inclusion criteria

All patients were between 0 and 18 years of age with different histological types of childhood cancers. All patients had no symptoms of infection (thus excluding any influence of inflammation). A complete blood count, C-reactive protein concentration, erythrocyte sedimentation rate, and liver and renal function tests were performed to verify lack of infection. None of the patients were receiving vitamin supplementation.

Control group

Thirty healthy children from the outpatient clinics were recruited as a control group (18 females and 12 males), between 1.4 and 17.9 years (mean age 9.75 years, median age 8.05 years). At the time of the study, on the basis of detailed medical history and clinical examination, all children were found to be in complete health with no contemporaneous disease. None of the participants were taking vitamin and/or antioxidant supplements for at least 8 weeks before the date of the study. The study was approved by the Ethical Committee of the Medical University of Gdansk, Poland.

Collection of samples

All assays were performed on samples from the patients before starting the first cycle of chemotherapy. Venous blood in the amount of 4 ml was taken from each patient and serum samples were centrifuged and stored at –70°C.

Laboratory methods

All assays were performed in the laboratory of the Department of Clinical Nutrition, Medical University of Gdansk, Poland.

The superoxide dismutase (SOD) assay in erythrocytes

SOD activity in erythrocytes was assessed according to the Ransod (Randox, Crumlin, United Kingdom) procedure. The increase of absorbance was followed at 505 nm for 3 min on a UV-VIS spectrophotometer (LKB Pharmacia, United Kingdom). Results were expressed in U/g Hb.

The glutathione peroxidase (GSH-Px) assay in erythrocytes

GSH-Px in erythrocytes was measured according to the Ransel (Randox, Crumlin, United Kingdom) procedure. The decrease of absorbance was recorded at 340 nm for 3 minutes. GSH was expressed in U/g Hb.

Assay of glutathione (GSH) in erythrocytes

GSH in erythrocytes was measured using a Sigma-Aldrich kit.

The final absorbance was measured at 400 nm. The glutathione was expressed in U/g Hb.

Ischemia modified albumin (IMA) in serum

The absorbance of assay mixtures was read at 470 nm with an LKB Spectrophotometer. The value of absorbance was given in units [U/ml serum] using ACB Roche calibrator of 221 U/ml as a standard.

Statistical analysis

The results obtained in the study were subjected to statistical analysis.

For continuous parameters mean ($\overline{X}$) and median (M) values, standard deviation (SD), range (min, max) and lower and upper quartile (25Q, 75Q) were calculated in all groups. Analysis of variance (ANOVA) was used to test the hypothesis of equality of means of particular trials. In groups with a low number of cases composite variance was assessed by means of a non-parametric Kruskal-Wallis sum of ranks test (Bartlett's test was used to check the homogeneity of variance). In all cases p values ≤ 0.05 were considered statistically significant. Statistical analysis was performed using EPI-INFO Ver.3.4.3 (08.-11-2007) software.

Results

Activity of oxidative markers

The activity of SOD was higher in STS, NB, and HD, but lower in the leukemias and brain tumor groups compared to the control group. Statistical significance was only observed in patients with brain tumors (median 1840.2 U/g Hb, $p = 0.0500$) (Fig. 1). Lower activity of GSH-Px was identified only in brain tumors but was not statistically significant. The level of GSH was significantly higher in patients with NB (median 6.38 U/g Hb, $p = 0.0031$) and leukemias (5.16 U/g Hb, $p = 0.0200$) (Figs. 2 and 3). In patients with HD, GSH was higher but was not statistically significant. GSH was decreased in patients with NB and STS but was not statistically significant. The level of IMA tended to be higher in all patients with neoplasms and was statistically significant in patients with STS, NB, and leukemias compared to healthy children ($p = 0.0244$, $p = 0.0069$, and $p = 0.0000$, respectively) (Fig. 4).

Discussion

A decrease of SOD activity was observed in pediatric patients with acute lymphoblastic (ALL) and acute non-lymphoblastic leukemia (ANLL) compared to control patients. Similar results were demonstrated by Gonzales *et al.* [10] in adult

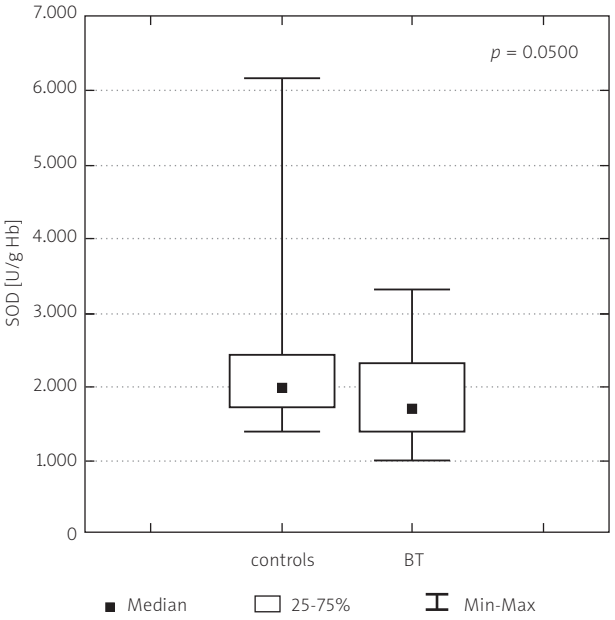

Fig. 1. Significantly higher activity of SOD in patients with brain tumor (BT) vs. control group (p = 0.0500)

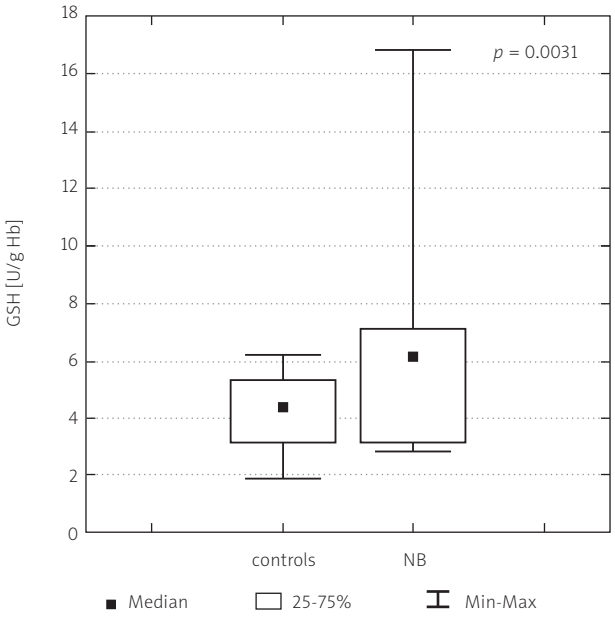

Fig. 2. Significantly higher level of GSH in patients with neuroblastoma (NB) vs. control group (p = 0.0031)

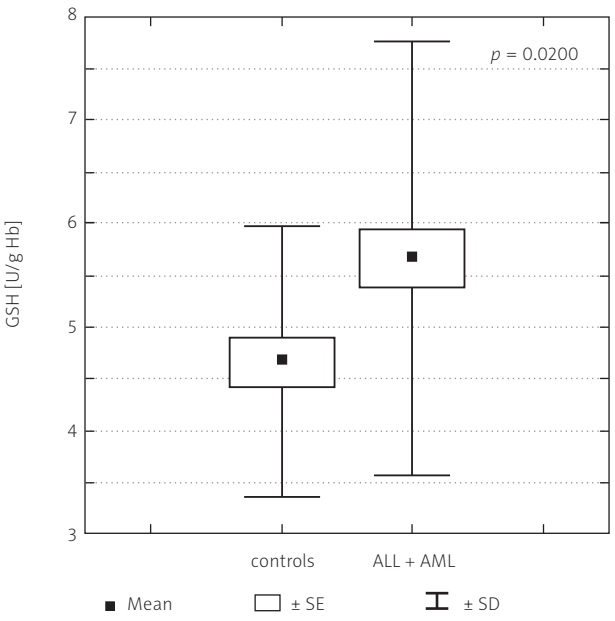

Fig. 3. Significantly higher level of GSH in patients with acute leukemias (ALL + AML) vs. control group (p = 0.0200)

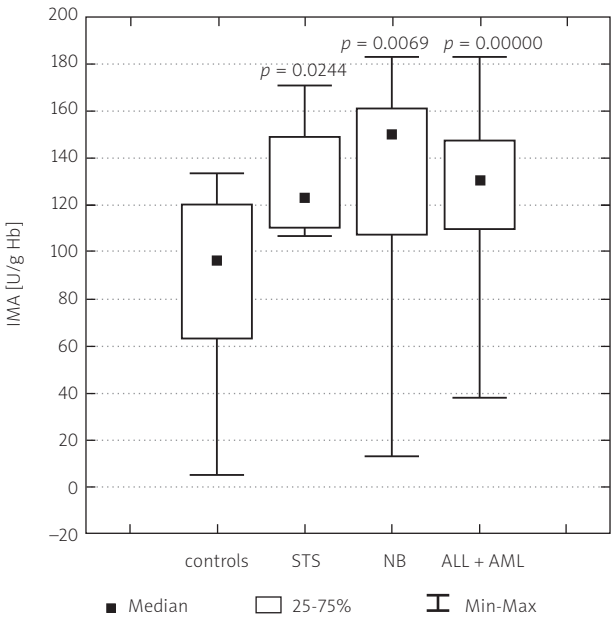

Fig. 4. Significantly higher level of IMA in patients with soft tissue sarcomas (STS), neuroblastoma (NB) and leukemias (ALL + AML) vs. control group (p = 0.0244, p = 0.0069, p = 0.0000)

patients with acute leukemias and HD. However, Jawniak *et al*. [11] described higher SOD activity in patients with ANLL prior to treatment. Another study reached the same conclusion but was performed in adult patients with other types of leukemia [12].

In pediatric patients with ALL, the oxidative stress increased during treatment, while the activity of serum GSH-Px decreased. The antioxidant status was closely connected with treatment-related oxidative stress. Supplementation with vitamin E and *N*-acetylcysteine in children with cancer may improve the therapeutic results [13].

Jawniak *et al*. [11] suggested that GSH-Px plays a prognostic role in patients with ANLL. Elevated levels of GSH-Px weaken the antioxidant potential, which is revealed by cytostatics, and may lower the chemosensitivity.

Adult patients with newly diagnosed leukemia exhibited lower GSH-Px activity compared to control patients. Studies have suggested that the lower activity may be the result of higher requirements during times of oxidative stress [14, 15]. Bewick *et al*. examined patients with lymphoma according to different histological types. Deficiencies in erythrocyte SOD and GSH-Px were observed, but no difference in enzyme ac-

tivity was noted between the different histopathological types of neoplasms [16].

Patients with brain tumors demonstrated a lower activity of both SOD and GSH-Px. Similar results were reported by Aggarwal *et al*. [17], who noted greater decreases in enzyme activity correlated with increases in the malignant histopathology. Rao *et al*. [18] analyzed 100 patients with different types of brain tumors and observed a significant decrease in erythrocyte SOD activity. Brain tissue is extremely sensitive to oxidative stress. The requirement for oxygen is much higher than in other tissues, and the antioxidant defense system is relatively poor [19]. A total of 24 adult patients with glioblastoma and astrocytoma were examined by Wozniak *et al*. [20]. Higher erythrocyte SOD activity was observed in these patients compared to the control group.

Decreased activity of GSH-Px was observed in children with solid tumors and HD compared to the control group [21]. Ordukhanian *et al*. [22] measured the antioxidant defense status in children with Wilms' tumor. A significant increase in SOD activity was observed in 85 patients prior to surgical intervention.

Kaya *et al*. [23] studied 34 adult patients with HD after 7 days of chemotherapy and observed a significant decrease in the activity of antioxidant enzymes, such as SOD and GSH-Px, and increased levels of free radicals. The overproduction of ROS in cancer cells may block the transcription of antioxidant enzyme genes, which increases the sensitivity to free radicals and leads to apoptosis.

Several reports demonstrate that SOD and GSH-Px decrease in various types of adult cancers [24, 25].

Currently, no reports have been identified that address the behavior of GSH and IMA in solid tumors and hematological malignancies in children. Our study group found significantly higher levels of GSH in children with NB and leukemia. Some authors have suggested that elevated GSH levels observed in adult patients with solid tumors may cause resistance to chemotherapy [26]. Decreased serum levels of GSH were observed in patients with oral squamous cell carcinoma. The possible explanation for this result may be increased scavenging of lipid peroxides, as well as the sequestration of GSH by tumor cells [24]. One study reported that tumor cells were able to sequestrate antioxidants, including GSH, that are essential in many processes in order to provide substrates for tumor growth [27].

IMA is a good prognostic marker for ischemic disorders [28]. One study comparing the levels of IMA between patients with benign prostate hyperplasia, prostate cancer, and myocardial infarction (MI) was identified. The IMA levels were higher in all measured groups, but the highest levels were observed in MI patients [9]. In our patients, the IMA levels were significantly higher in patients with STS, NB, and leukemia. Stachowicz *et al*. [29] measured the levels of IMA in pediatric patients with STS and NB before and after treatment. The levels of IMA were much higher before treatment than during therapy and remained elevated compared to levels in the control group.

In conclusion, we observed that the antioxidant barrier in all types of pediatric cancers is disturbed. Our study showed that none of the measured parameters is specific enough to become a reliable marker for any particular histopathological type of children's neoplasm.

All authors confirm that they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

No authors declare any potential conflicts of interest.

The funding organizations played no role in the design of the study, selection of enrolled patients, review and interpretation of data, or preparation or approval of the manuscript.

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Submitted: 18.08.2011
Accepted: 18.01.2012