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Impact of the hyperbaric oxygen therapy on the redox status in the patients with systemic lupus erythematosus

Uticaj hiperbarične oksigenoterapije na redoks status bolesnika sa sistemskim eritemskim lupusom

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Abstract

Background/Aim. Hyperbaric oxygen therapy (HBOT) is a method which increases oxygen solubility in plasma up to 20 times. This effect is very important in the treatment of circulatory disorders, which reduces oxygenation and leads to increased production of inflammatory mediators and free oxygen radicals. The aim of this study was to examine the impact of HBOT on the oxidative stress parameters in the patients with systemic lupus erythematosus (SLE). **Methods.** This prospective study included 18 females with SLE [American College of Rheumatology (ACR) criteria], average age 52.2 ± 8.82 years, treated with HBOT for 60 min/day, with average partial oxygen pressure of 2.2 atmospheres absolute (ATA), during 10 days, in combination with appropriate medication therapy for SLE. The following parameters were determined in the serum: C-reactive protein (CRP), hemoglobin, creatinine, albumin, complement 3 (C3), antinuclear antibodies (ANA), glomerular filtration rate (GFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula. In the urine, parameters of oxidative stress were spectrofotometrically determined: levels of superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), nitrites (NO_2^-) and concentration of thiobarbituric acid reactive substances (TBARS). In hemolysate, the parameters of antioxidant protection: superoxide dismutase (SOD), catalase (CAT) and reduced gluthatione (GSH), were measured. The samples for the analysis were collected three times: before HBOT (initial values), after 10 days of HBOT and 1 month after beginning the treatment in relation to the initial value. **Results.** We noticed a statistically significant ($p < 0.05$) decrease in a level of $O_2^{\bullet-}$, both after 10 days and after 1 month of HBOT (8.26 ± 13.62; 8.39 ± 4.94; 11.92 ± 6.86 nmol/mL, respectively). Values of other parameters of oxidative stress such as NO_2^-, TBARS and H_2O_2 showed no significant difference during the monitored period. Regarding the parameters of antioxidant the protection, we revealed slightly higher value of GSH after treatment (initial value: 66.34 ± 16.31; after 10 days of HBOT 79.43 ± 36.77; after 1 month of HBOT69.72 ± 22.32 μmol/mL red blood cells) which was held after a month, but it was not statistically significant. Activity of SOD and CAT, before and after HBOT, did not change significantly. **Conclusion.** Our results suggested the potential beneficial effects of HBOT on redox status in the patients with SLE by decreasing the levels of $O_2^{\bullet-}$.

Key words:
hyperbaric oxygenation; lupus erythematosus; oxidation-reduction; oxidative stress.

Apstrakt

Uvod/Cilj. Hiperbarična oksigenoterapija (HBOT) je metoda kojom se rastvorljivost kiseonika u plazmi povećava i do 20 puta. Taj efekat je veoma značajan u terapiji poremećaja cirkulacije koji smanjuju oksigenaciju i dovode do povećanja produkcije medijatora zapaljenja i slobodnih kiseoničkih radikala. Cilj ove studije bio je da se ispita uticaj HBOT na parametre oksidativnog stresa kod bolesnika sa sistemskim eritemskim lupusom (SLE). **Metode.** Prospektivnom studijom obuhvaćeno je 18 bolesnica sa SLE (kriterijumi Američkog koledža za reumatologiju) prosečne starosti 52,2 ± 8,82 godina, koje su tretirane HBOT u trajanju od 60 min/dan, pri pritisku od 2,2 apsolutne atmosfere (ATA),

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ukupno 10 dana, u kombinaciji sa odgovarajućom terapijom za SLE. U serumu su određivani sledeći parametri: C-reaktivni protein (CRP), hemoglobin, kreatinin, albumin, komplement 3 (C3), antinuklearna antitela (ANA), stopa glomerularne filtracije (GFR korišćenjem *Chronic Kidney Disease Epidemiology Collaboration* (CKD-EPI) formule. U urinu su spektrofotometrijski određivani parametri oksidacionog stresa: nivo superoksid anjon radikala ($O_2^{\bullet-}$), vodonik peroksida (H_2O_2), nitrita (NO_2^-) i koncentracija reaktivnih produkata tiobarbituratne kiseline (TBARS). U hemolizatu, određivani su parametri antioksidativne zaštite: aktivnost superoksid dismutaza (SOD), katalaze (CAT) i redukovani glutation (GSH). Uzorci za analize su sakupljani tri puta: pre HBOT (inicijalne vrednosti), nakon 10 dana HBOT i nakon mesec dana terapije. **Rezultati.** Uočili smo statistički značajno ($p < 0,05$) smanjenje nivoa $O_2^{\bullet-}$ nakon 10 dana, kao i nakon mesec dana od početka HBOT u odnosu na inicijalnu vrednost (8,26 ± 13,62; 8,39 ± 4,94 11,92 ± 6,86; nmol/mL, redom). Nisu pokazane značajne razlike u vrednostima ostalih parametara oksidativnog stresa kao što su NO_2^-, TBARS i H_2O_2 tokom posmatranog perioda. Što se tiče parametara antioksidativne zaštite, otkrili smo nešto veću vrednost GSH nakon tretmana (inicijalno 66,34 ± 16,31; posle 10 daba 79,43 ± 36,77; posle mesec dana tretmana 69,72 ± 22,32 μmol/mL eritrocita), koja se održala nakon mesec dana, ali nije bila statistički značajna. Aktivnost SOD i CAT, pre i posle HBOT, nije se statistički značajno menjala. **Zaključak.** Naši rezultati ukazuju na povoljan efekat HBOT na redoks ravnotežu kod bolesnika sa SLE zbog sniženja nivoa $O_2^{\bullet-}$.

Ključne reči:
hiperbarična oksigenacija; lupus, eritematozni, sistemski; oksidoredukcija; stres, oksidativni.

Introduction

Systemic lupus erythematosus (SLE) is a very serious autoimmune inflammatory disease, with an unpredictable course and outcome, whose etiology remains largely unknown and the effects of conservative treatment are limited [1]. Human and animal studies indicate that oxidative stress is involved in the pathogenesis of SLE. Excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), including peroxynitrite- $ONOO^-$, can damage lipids, proteins and DNA and products of oxidative modification can be detected in biological fluids [2]. The abundance of those products correlates with disease activity in the SLE patients, suggesting oxidative modification acts as a biomarker [3–5]. While several studies implicate nitric oxide as an important mediator of disease in the SLE [6, 7], there is a lack of data revealing the association between the level of urine nitrite and citrulline levels, as surrogate markers of nitrogen monooxide (NO) production, and disease activity among the patients with SLE [8]. Also, previous data suggested that lipid peroxidation could be a risk factor for endothelial dysfunction in some autoimmune diseases [9].

Hyperbaric oxygen therapy (HBOT) is a treatment modality in which a person breathes 100% O_2 intermittently while exposed to increased atmospheric pressure, greater than 1 atmosphere, absolute (ATA) usually 2 to 2.5 ATA [10]. The primary mechanisms of action include hyperoxygenation and a decrease in bubble size, or vasoconstriction, angiogenesis, fibroblast proliferation, oxidative leukocyte degradation, toxin inhibition and antibiotic synergy [11, 12]. Hyperbaric oxygen may be used as the primary therapy intervention in some conditions, such as carbon monoxide poisoning, decompression sickness and arterial gas embolism, arterial insufficiencies, cardiovascular diseases, osteomyelitis and as an adjunctive therapy for wound healing [13–15]. The HBOT showed to have the beneficial effects on hypoxic diabetic ulcers that result in severe wound-healing problems and osteoradionecrosis and is frequently used for necrotic soft tissues and bone that fails to heal. The HBOT also induces significant angiogenesis, which in one study was measurable after the eight HBOT sessions. Previous clinical studies revealed that vasculitis skin ulcers in the patient suffering from SLE was treated successfully with the HBOT [16–18]. There are not many studies that examined effects of HBOT on redox homeostasis and inflammation in the patients with SLE.

Given the fact that the HBOT can modify oxidation-reduction reactions, the aim of our study was to establish an influence of hyperbaric oxygenation on the oxidative stress parameters and antioxidant enzymes activity in the patients with SLE.

Methods

This prospective study included 18 females with SLE, treated with the HBOT once a day for 60 min (total 10 days) with the average partial oxygen pressure of 2.2 ATA, in combination with an appropriate therapy for SLE. The study protocol was approved by the Institutional Ethics Committee (Military Medical Academy Belgrade, Serbia) and the study was conducted in accordance with the Declaration of Helsinki. All the participants were informed about the research protocol before giving their written consent to participate in the study.

All patients were admitted to the Military Medical Academy, Belgrade, Serbia from October 2011 to December 2014, with: a diagnosis of SLE. In order to define a severity of the disease course in this study, the original 1997 American College of Rheumatology (ACR) classification of SLE was used [18, 19]. At the beginning of study, all participants were in the similar stage of the disease, in remission [Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score 0.30 ± 0.47]. The exclusion criteria were: the pregnant women with SLE, the patients with urinary infection (positive urine culture), with renal insufficiency (creatinine clearance < 60 mL/min), the presence of malignancy, the patients with any other ongoing inflammatory process, or under 18 years of age. The patients who were on immunosuppressive therapy such as mycophenolate mofetil, cyclophosphamide and other cytotoxic agents, were excluded. The only therapy, that the patients took, was the corticosteroids (the maintenance dose of 5 mg).

All patients with any contraindication for the HBOT were also excluded. All participants were non-smokers and did not take any antioxidant dietary supplement for 1 month before the study. Before beginning the HBOT, all participants passed a standard medical and physical revision at the hospital. During the study period no patient was eliminated.

Hyperbaric oxygen therapy

The HBOT was performed at The Center for Hyperbaric Medicine, Military Medical Academy in Belgrade, Serbia. The HBOT consisted of 10 sessions (1 session a day/5 days a week) in a multiplace (10-person) hyperbaric chamber**.** In total 60 min of 100% medical oxygen was administered to the patients under the increased pressure of 2.2 ATA during a 70-min hyperbaric session. At this pressure, 100% oxygen was delivered via an oronasal mask in two episodes of 30 min, each interrupted by 5 min of air breathing. During the pressure changes, great care was taken to avoid barotraumas, particularly of the middle ear, which is the most common side-effect of a hyperbaric treatment. All patients tolerated the treatment well without any complications.

Biochemical analysis

The samples for biochemical analysis were collected three times: before the HBOT (initial values), after 10 days of the HBOT (2 hours after the last HBOT session) and after 1 month. The following parameters were determined in the blood serum samples: C-reactive protein (CRP), hemoglobin (Hb), creatinine, albumin, complement 3 (C3), antinuclear antibodies (ANA), glomerular filtration rate (GFR using the CKD-EPI formula). In the urine samples, the following parameters of the redox status were spectrofotometrically determined: levels of superoxide anion radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), nitrites (NO_2^-) and concentration of thiobarbituric acid reactive substances (TBARS). The parameters of antioxidant protection were measured in the blood samples: activity of superoxide dismutase (SOD) and catalase (CAT) and level of reduced glutathione (GSH).

Superoxide anion radical determination

The level of $O_2^{\bullet-}$ was measured using nitro blue tetrazolium (NBT) reaction in TRIS-buffer combined with the urine samples and read at 530 nm [20].

Hydrogen peroxide determination

The protocol for measurement of H_2O_2 is based on oxidation of phenol red in the presence of horseradish peroxidase [21]. The 200 μL sample with 800 μL of phenol red solution (PRS) and 10 μL of Horseradish Peroxidase (HRP) were combined (1 : 20). The level of H_2O_2 was measured at 610 nm.

Nitric oxide determination

Nitric oxide (NO) decomposes rapidly to form the stable metabolite nitrite/nitrate products. NO_2^- was determined as an index of nitric oxide production with the Griess reagent [22]. 0.1 mL of 3 N Perchloride acid (PCA), 0.4 mL 20 mM ethylenediaminetetraacetic acid (EDTA), and 0.2 mL urine were put on ice for 15 min, then centrifuged 15 min at 6,000 rpm. After pouring off the supernatant, 220 μl of K_2CO_3 was added. Nitrites were measured at 550 nm. Distilled water was used as a blank probe.

Determination of concentration of thiobarbituric acid reactive substances

The degree of lipid peroxidation in urine was estimated by measuring concentration of TBARS using 1% thiobarbituric acid (TBA) in 0.05 NaOH, incubated with urine at 100 °C for 15 min and read at 530 nm. Distilled water was used as a blank probe. The TBA extract was obtained by combining 0.8 mL of urine and 0.4 mL of trichloro-acetic acid (TCA), then the samples were put on ice for 10 min and centrifuged for 15 min at 6,000 rpm. This method was described previously [23].

Preparation of hemolysate

The blood samples were taken from the antecubital vein into vacutainer test tube containing sodium citrate anticoagulant. Blood was centrifuged to separate plasma and red blood cells (RBCs). Isolated RBCs were washed 3 times with 3 vol. of ice cold 0.9 mmol/L NaCL. The blood samples were stored immediately and kept for further analyses [24].

Determination of antioxidant enzymes catalase and superoxide dismutase

Hemolysates containing about 50 g Hb/L prepared according to McCord and Fridovich [24] were used to determine the CAT activity which was expressed in U/gHb × 1,000. The CAT activity was determined according to Beutler [25]. Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove hemoglobin. Then 50 μL of CAT buffer, the 100 μL of sample, and 1 mL of 10 mM H_2O_2 were added to the samples. Detection was performed at 360 nm. Distilled water was used as a blank probe. The SOD activity was determined by the epinephrine method of Misra and Fridovich [26] and it was expressed in U/gHb × 1,000. A hundred μL of lysate and 1 mL carbonate buffer were mixed, and then 100 μL of epinephrine was added. Detection was performed at 470 nm.

Determination of reduced glutathione

A level of GSH was determined spectrophotometrically, and it was based on GSH oxidation via 5,5-dithiobis-6,2-nitrobenzoic acid. The GSH extract was obtained by combining 0.1 mL 0.1 % EDTA, 400 μL haemolysate, and 750 μL precipitation solution (containing 1.67 g metaphosphoric acid, 0.2 g EDTA, 30 g NaCl, and filled with distilled water until 100 mL; the solution was stable for 3 weeks at +4 C°). After mixing in the vortex machine and extraction on cold ice (15 min), it was centrifuged on 4,000 rpm (10 min). Distilled water was used as a blank probe. Measuring was performed at 420 nm. The concentration is expressed as micromoles per mL of RBCs [25, 27].

Statistical analysis

In case of continuous data, the variables were presented as the mean value ± standard deviation (SD). The Kolmogorov-Smirnov test was used for evaluation of distribution of biochemical data. A statistical significance between the groups was tested by the Friedman (repeated measure) test (post hoc Wilcoxon test). All the analyses were estimated at $p < 0.05$ level of statistical significance. A complete statistical analysis of data was done by the statistical software package, SPSS Statistics 18.

Results

A total of 18 women, the average age 52.22 ± 8.82 years, were enrolled in the study. The patients presented SLE with an average time without symptoms of healing of 20.2 ± 5.0 months when they underwent the HBOT.

The values of the serum parameters such as CRP, Hb, creatinine, albumim, C3, ANA, chronic kidney disease estimated (CKD eGFR), were not statistically significantly different when compared the initial values, with the values after 10 days and after a month of the therapy (Table 1).

Levels of superoxide anion radical

We noticed a statistically significant decreased ($p < 0.05$) levels of $O_2^{\bullet-}$ after 30 days of the HBOT beginning compared to the initial values of this parameter, and significantly decreased values after 10 days of the HBOT compared to the initial values (11.92 ± 6.86, 8.26 ± 13.6, 8.39 ± 4.94 nmol/mL, respectively) (Figure 1).

Table 1

Comparison of selected laboratory parameters - the initial value, value after 10 days and after a month of HBOT

Laboratory parameters in serum	Initial (mean ± SD)	10th day (mean ± SD)	30th day (mean ± SD)
CRP (mg/L)	5.16 ± 5.7	4.93 ± 6.06^{ns}	3.26 ± 2.25^{ns}
Hb (g/L)	129.3 ± 10.27	131.00 ± 12.51^{ns}	129.44 ± 14.13^{ns}
Creatinine (mmol/L)	72.00 ± 22.84	78.55 ± 26.09^{ns}	74.55 ± 23.55^{ns}
Albumin (g/L)	42.33 ± 2.95	40.66 ± 2.50^{ns}	42.33 ± 2.95^{ns}
C3 (g/L)	1.05 ± 0.23	1.05 ± 0.29^{ns}	1.03 ± 0.25^{ns}
ANA (IU/mL)	1.11 ± 1.16	1.44 ± 1.33^{ns}	1.00 ± 1.11^{ns}
CKDeGFR (mL/min/1.73 m^2)	89.00 ± 22.46	84.33 ± 19.53^{ns}	87.44 ± 20.91^{ns}

^{ns} non significant differences in comparison to the initial values
HBOT – hyperbaric oxygen therapy; CRP – C-reactive protein; Hb – hemoglobin; C3 – complement 3; ANA – antinuclear antibody; (CKD eGFR) – a chronic kidney disease estimated glomerular filtration rate.

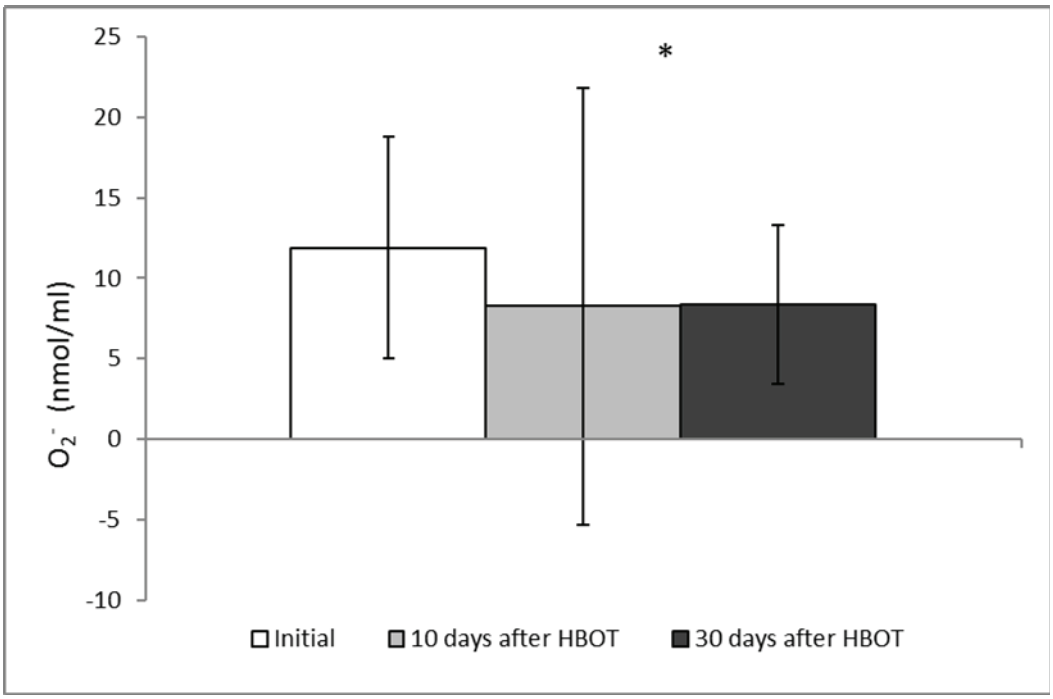

Fig. 1 – Level of superoxide anion radical (O2-) in the urine samples (the values are presented as mean and standard deviation). The statistical significances are presented as a significance between the values after 10 days vs. initial values and after 30 days of hyperbaric oxygen therapy (HBOT) vs. the initial values (*p < 0.05).

Levels of nitrites

The levels of NO_2^- before and after the HBOT were similar in all terms observed (2.72 ± 0.16, 2.77 ± 0.29, and 2.76 ± 0.20 nmol/mL, respectively). We found that this parameter was not significantly affected by the HBOT when comparing the initial values to the values after 10 days of the HBOT and 1 month after the treatment begining (Figure 2).

Levels of hydrogen peroxide

During the observed period of HBOT, there were no statistically significant changes of H_2O_2 levels when compared the initial values, the values after 10 days and after 1 month of the HBOT. This parameter was not changed during the study (1.52 ± 0.08, 1.51 ± 0.07, 1.54 ± 0.17 nmol/mL, respectively) (Figure 3).

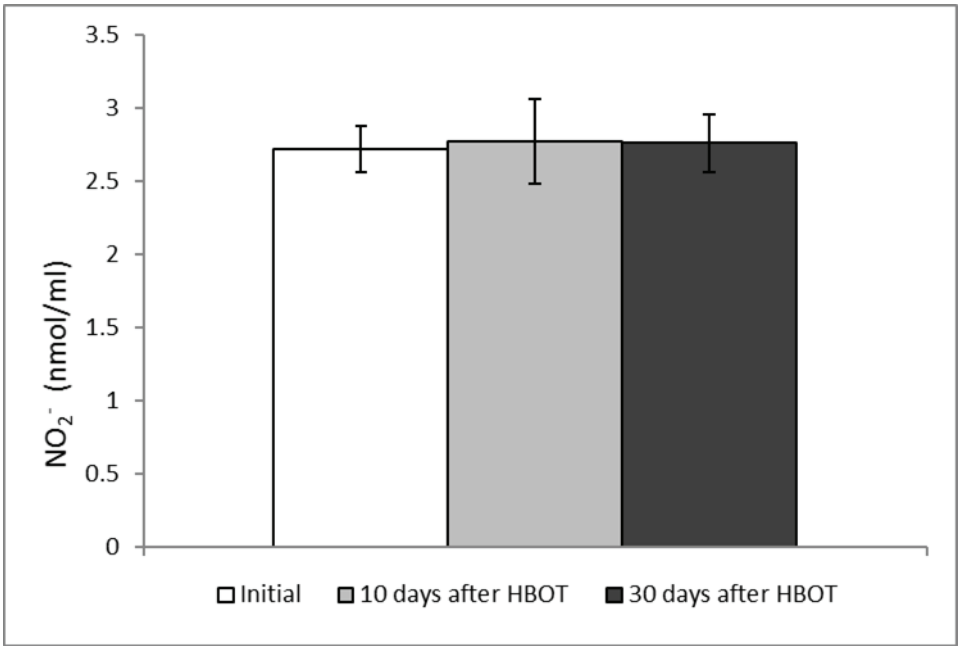

Fig. 2 – Levels of nitrites (NO_2^-) in the urine samples (the values are presented as mean and standard deviation). There was no significant difference before and after the hyperbaric oxygen therapy (HBOT).

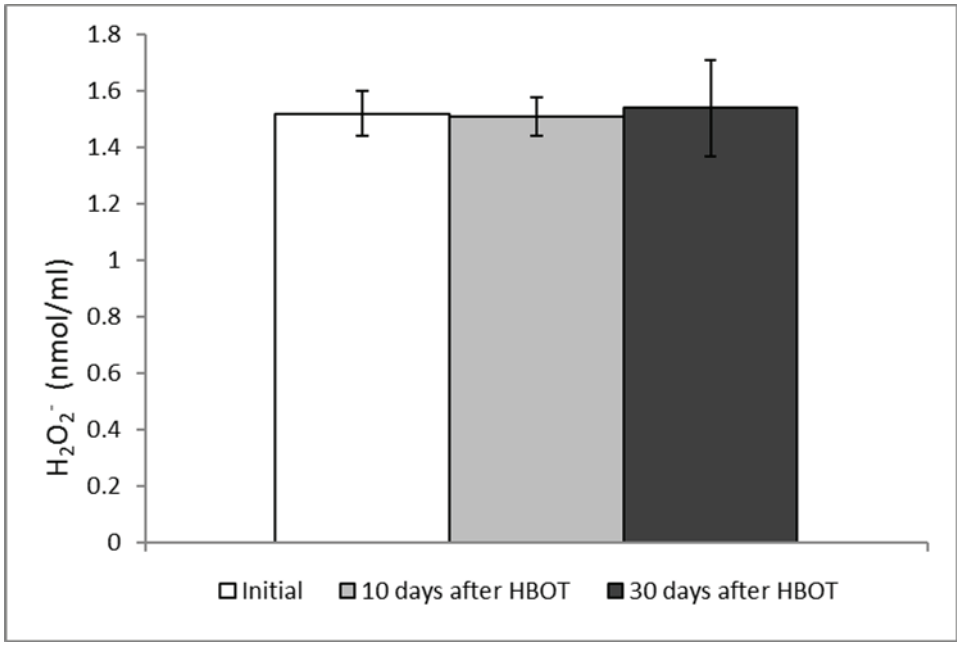

Fig. 3 – Levels of hydrogen peroxide (H_2O_2) in the urine samples (the values are presented as mean and standard deviation). There were no significant differences before and after the hyperbaric oxygen therapy (HBOT).

Concentration of TBARS

Among the examined groups, TBARS concentration was not significantly altered suring the study (1.01 ± 0.11; 1.09 ± 0.11; 1.05 ± 0.96 μmol/mL, respectively) (Figure 4).

Activity of superoxide dismutase (SOD)

In the study group, we noticed a decreased activity of SOD after the HBOT when compared the initial value to the value after 10 days of the therapy (27.58 ± 8.86, and 19.47 ± 10.63 U/gHb×10^3, respectively). However, a month after, the values were similar to those from the beginning of the study protocol and the activity was 27.11 ± 28.26 U/gHb×10^3. Those changes were without a statistical significance (Figure 5).

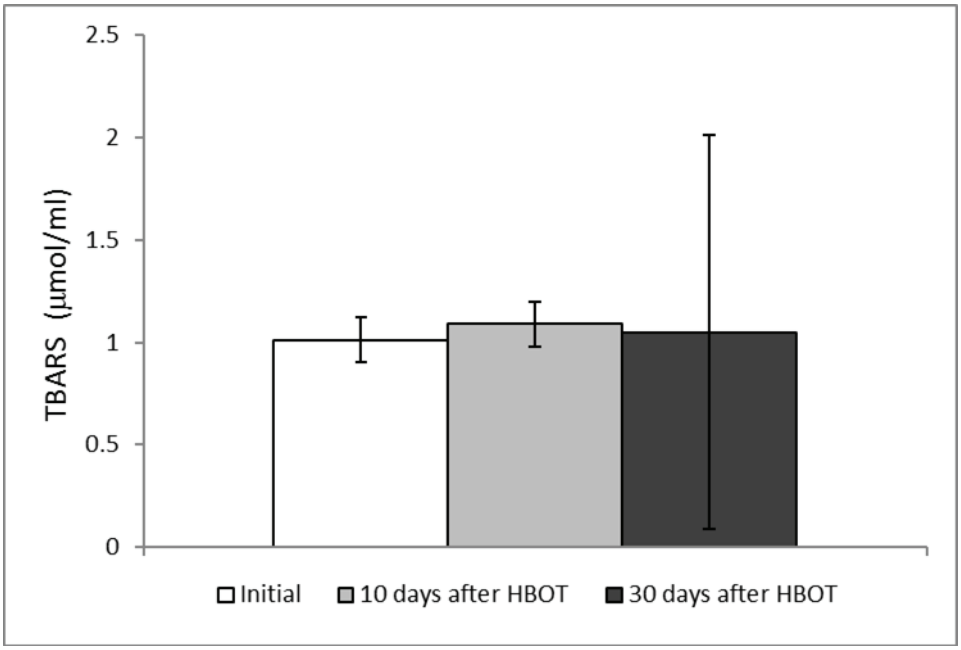

Fig. 4 – Concentration of TBARS in the urine samples (the values are presented as mean and standard deviation). There were no significant differences before and after the hyperbaric oxygen therapy (HBOT).
TBRS – thiobarbituric acid reactive substances.

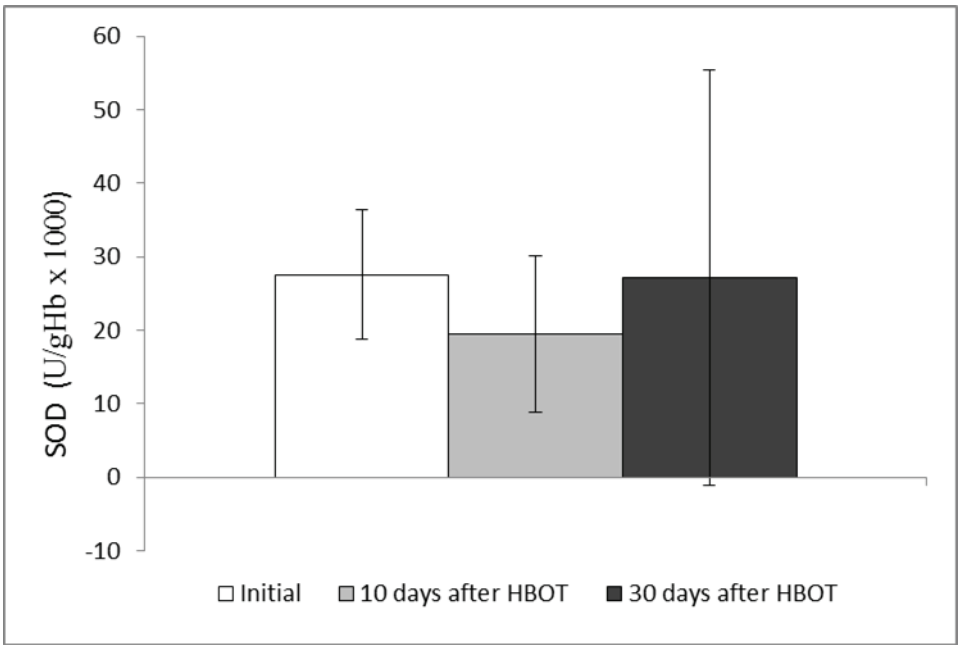

Fig. 5 – Activity of superoxide dismutase (SOD) in the blood samples (the values are presented as mean and standard deviation). Comparing the initial values of SOD and those after the hyperbaric oxygen therapy (HBOT), it was observed decrease in the level, but without a significant difference. After 30 days activity of SOD is similar to the initial values.

Level of reduced glutathione

A level of GSH was not statistically significantly increased in our group (initially 66.34 ± 16.31, 79.43 ± 36.77 μmol/mL RBC after the HBOT). However, a month after values were similar to those from the beginning of the study protocol and the activity was 69.72 ± 22.32 μmol/mL RBC (Figure 6).

Activity of catalase

We observed a decrease in the activity of CAT after 10 days of the HBOT compared to the initial value (4.77 ± 2.93 and 5.44 ± 3.55 U/gHb×10^3, respectively), and it continued to decrease, so after a month it was lower than before the HBOT and after 10 days of the HBOT (3.82 ± 2.49 U/gHb×10^3, respectively). However those differences were not statistically significant (Figure 7).

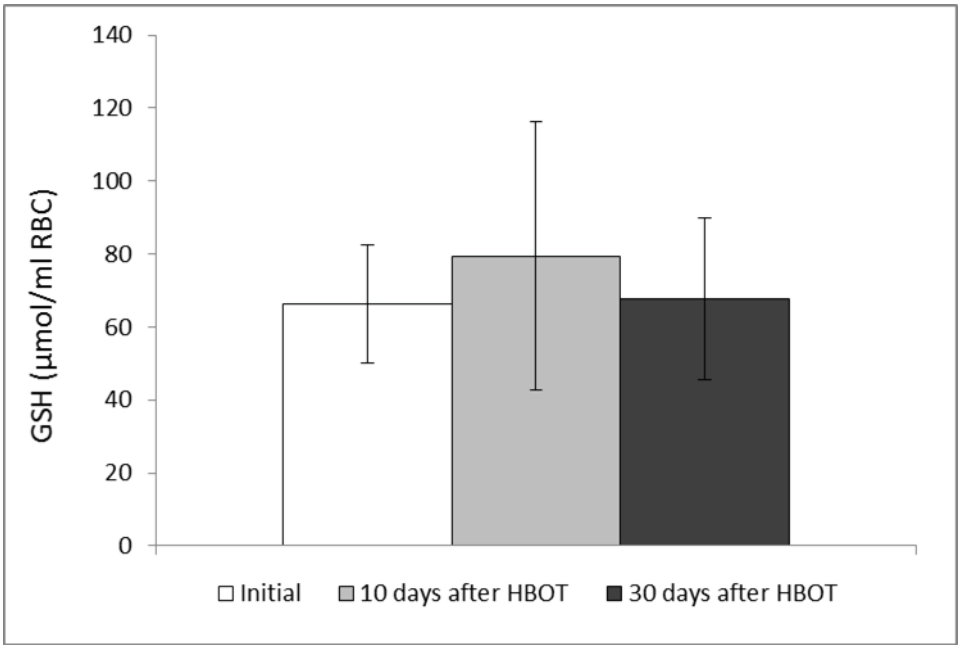

Fig. 6 – Level of reduced glutathione (GSH) in the hemolysate samples (the values are presented as mean and standard deviation). Comparing the values of GSH initially and after the hyperbaric oxygen therapy (HBOT)] there were no statistically significant differences.

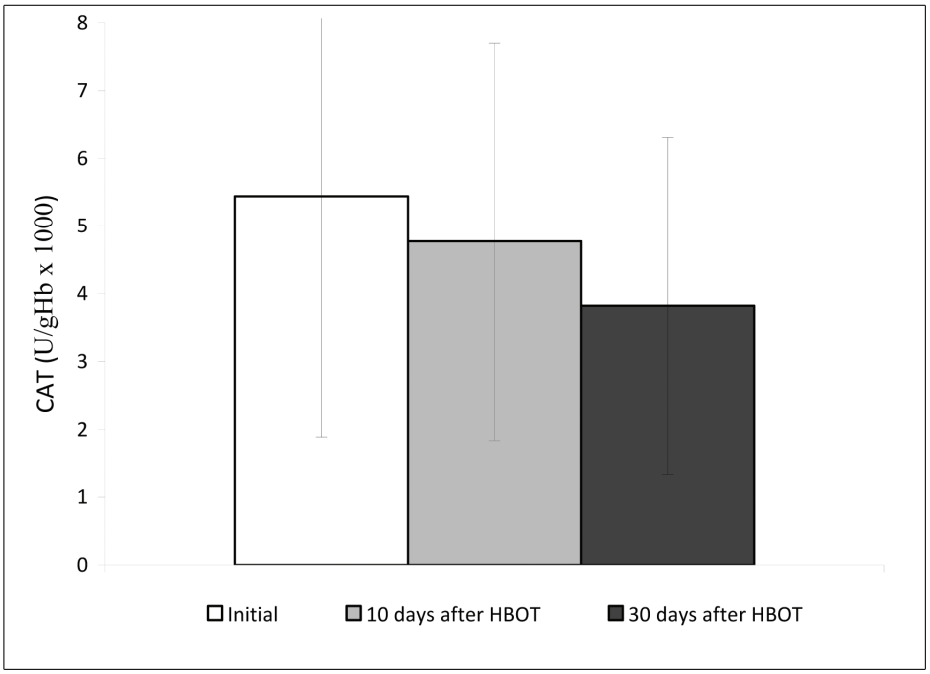

Fig. 7 – Activity of catalase (CAT) in the hemolysate samples (the values are presented as mean and standard deviation). Comparing the initial values and those after 10 days and 30 days, the hyperbaric oxygen therapy (HBOT), there were no statistically significant differences.

Discussion

This study was designed in the field of physiology research of hyperbaric oxygenation with a special emphasis on potential systemic effects of disturbed redox balance, induced by a systemic disease before and after the application of oxygen. Actually, the HBOT leads to an increase in the amount of dissolved oxygen in plasma, creating a diffusion gradient which facilitates the transition of oxygen from the capillaries to the ischemic tissues [17]. Studies reported controversial results regarding the effect of the HBOT on the oxidative stress and enzymes of antioxidative defense in the several pathophysiological models. The role of ROS and RNS in therapeutic responses of the HBOT in the patients with SLE has still not been completely revealed and explained [28–31].

Immune dysfunction, genetic, hormonal and environmental factors are included in an etiology of SLE, however molecular mechanisms underlying this systemic autoimmune response remain largely unknown [31, 32]. It is believed that the oxidative stress has an important role in the pathogenesis of SLE. Excessive production of ROS (including $ONOO^-$) can damage all biomolecules such as lipid, protein and DNA and cause a formation of different products which can be detected in biological fluids [2–5]. In case of the patients with SLE, this fact can be useful since their abundance correlates with the disease activity and organ damage [3]. Our study included the patients with SLE in whom the disease was in remission, which was maintained before and after the HBOT was applied. Comparing the laboratory parameters (CRP, hemoglobin, creatinine, albumin, complement C3, ANA) before and after the performed therapy as well as after a month, we did not notice the statistically significant differences in their values. We examined the effects of 10 session of the HBOT on the parameters of redox balance in the patients with SLE. We found the statistically significant decreased levels of $O_2^{\bullet-}$ after the HBOT, which were held after 30 days. There is a concern that the HBOT might increase the oxidative stress via the production of reactive oxygen species, however, the oxidative stress appears to be less of a concern at the hyperbaric pressures under 2.0 ATA [33]. The patients in our study were exposed to the higher pressure such as 2.2 ATA and we revealed the beneficial effects of hyperbaric oxygen on O_2^- levels. On the other hand, other pro-oxidants, such as NO_2^-, TBARS and H_2O_2 were not affected by the HBOT. In order to validate our results, we excluded all patients with renal disease or urinary infection, because the oxidative stress parameters may have not been removed from plasma because of insufficient excretion and may continue to rediffuse in circulation [30–34]. So, because of this fact, we could not be sure in unchanged levels of the oxidative markers.

Literature data regarding the effects of the HBOT on SLE is limited, and it is hard to compare our results to the others due to the fact that available researches were mostly focused on the effects of the HBOT on ulcers healing. One of a few studies which examined the effects of the HBOT in a SLE patient was a case report conducted by Olivieri et al [17]. They described the SLE patient with a case of refractory vasculitic ulcer responding to the HBOT, which was used in combination with immunosuppressive therapy. Jou et al. [35] reported their experience with the use of the HBOT for the treatment of intractable hemorrhagic cystitis in a SLE patient treated with cyclophosphamide. They concluded that this treatment was very successful, with no recurrent hematuria after the HBOT during 6 months [35]. Efrati et al. [16] reported that the HBOT may serve as an effective safe treatment for the patients with vasculitis having nonhealing skin ulcers, which is in agreement with the results of previously mentioned authors as well as with ours regarding a safety of the HBOT. The increase in tissue oxygenation appeared to be one of the major components responsible for the high cure rates in the patients with ulcers [16, 17].

In order to complete our picture about the influence of HBOT on redox status, we examined the activity of the antioxidant enzyme system. GSH is an important endogenous antioxidant and prime scavenger of free radicals in cells. One of the body's most powerful natural antioxidant enzymes are SOD and CAT. SOD, essential to catalyze the dismutation of superoxide, has been shown to protect cells from oxygen free radicals. Exposure to ROS from a variety of sources led to development of a series of defence mechanisms to neutralize these species and so protect cells against their toxic effects and that protection is achieved mainly by enzymatic antioxidants such as catalase. Some research conclude that hyperbaric oxygen treatment below 2.0 ATA can increase the activity of antioxidant enzymes including SOD, GSH and CAT [36–40].

Regarding a component of antioxidant defense including SOD, GSH, CAT we observed that the levels of GSH were higher (but without statistical significance) after 10 days exposed to hyperbaric oxygen treatment and a month later, too.

We believe that these beneficial results in regard to levels of $O_2^{\bullet-}$ and GSH after the HBOT imply the possibility that the study with a lager number of patients or changes of number of treatments could have results which would be statistically significant for these parameters. That refers to results noticed for SOD and CAT.

Activities of SOD and CAT were affected by the HBOT, but not statistically significantly. We noticed that the activity of SOD decreased after 10 days, but returned to the initial level after 30 days, and level of CAT decreased after a month compared to the initial value and value after 10 days of the HBOT. However, these changes were not statistically significant. Considering that differences in the activity of SOD and CAT between the peroxide initial level and the level after the HBOT were insignificant, we did not observe any significant influence on prooxidants such as hydrogen.

We believe that smaller number of patients in our study influenced our results. The assumption is that the future studies of a different design (larger number of patients, more treatments, additional analysis, etc.) could clarify the fact that we did not get a correlation between the decreased level of O_2^- (statistically significant in our study) and the increased values of the antioxidant protection parameters (GSH was not statistically significantly changed in our study).

We decided to treat the SLE patients with the HBOT not only because it improves the oxygenation of ischemic

tissues and exerts the beneficial effects on vascular inflammatory response by regulating the chemotaxis of leukocytes, but also because it facilitates the healing process of infected wounds promoting the deposition of collagen, angiogenesis, epithelialization and facilitating the oxygen-dependent killing by leukocytes [16–18]. Previous studies suggested that vasculitis skin ulcers in the patient suffering from SLE had been treated successfully with the HBOT [17].

Given the fact that the HBOT can modify oxidation-reduction reactions and because of the mentioned beneficial effects of the HBOT in different tissues in the patients with SLE, this protocol of therapy can be one of the possibility.

Conclusion

Our results highlighted some of the beneficial effects of hyperbaric oxygen treatment on redox balance among the patients suffering from SLE. However, the management of SLE is complex and more research is required to establish the complete mechanism by which the HBOT can modify oxidation-reduction reactions in the patients with SLE, so it can become an additional potential therapeutic strategy in the treatment of SLE.

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