Ann. Acad. Med. Siles. (online) 2018; 72: 116–120
eISSN 1734-025X
DOI: 10.18794/aams/76443

PRACA ORYGINALNA
ORIGINAL PAPER

Evaluation of antioxidant status in selected connective tissue diseases

Ocena stanu antyoksydacyjnego w wybranych chorobach układowych tkanki łącznej

Brygida Przywara-Chowaniec[1], Sebastian Seget[2], Maria Dróżdż[2], Agata Puzio[1], Zenon Czuba[2], Ewa Nowalany-Kozielska[1], Damian Kawecki[1], Maciej Gawlikowski[3]

[1]2nd Department of Cardiology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Katowice, Poland
[2]Department of Microbiology and Immunology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Katowice, Poland
[3]Professor Zbigniew Religa Foundation of Cardiac Surgery Development

ABSTRACT

INTRODUCTION: Reactive oxygen species (ROS), which cause increased degradation of nitric oxide and defects in endothelial function, play an essential role in the pathogenesis of increased vascular contractility. Reactive oxygen species are produced in the vascular bed by endothelial cells, smooth muscle cells and fibroblasts. The destructive effect of ROS is of great importance to the pathogenesis of many diseases such as atheromatosis, ischemia, heart failure, cerebral insufficiency and respiratory failure.
MATERIAL AND METHODS: A total of 36 patients (31 females, 5 males; mean age 47 ± 7 years) with connective tissue diseases (systemic lupus erythematosus – 23 patients, systemic sclerosis – 13 patients) and 40 (37 females, 3 males) healthy volunteers (mean age 50 ± 2 years) of both sexes were enrolled in the study. This study evaluated the total antioxidant capacity in serum, using the method of reducing radical DPPH and radical $ABTS^{\bullet+}$.
RESULTS: The blood plasma of the patients had lower antioxidant activities compared to the blood plasma of the healthy controls.
CONCLUSIONS: This study shows the differences in the antioxidant activity in healthy volunteers and patients with connective tissue diseases. These parameters can be monitored during treatment by modulating oxidative metabolism and by stimulating antioxidant activity.

KEY WORDS
connective tissue diseases, oxidative stress, superoxide dismutase

Received: 02.02.2017 **Revised:** 09.02.2017 **Accepted:** 16.08.2017 **Published online:** 20.06.2018

Address for correspondence: Dr n. med. Brygida Przywara-Chowaniec, 2nd Department of Cardiology, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Katowice, ul. M. Curie-Skłodowskiej 10, 41-800 Zabrze, Poland, tel. + 48 603 981 767, e-mail: bpzabrze@poczta.onet.pl

STRESZCZENIE

WSTĘP: Reaktywne formy tlenu zwiększają degradację tlenku azotu i upośledzają funkcję śródbłonka, odgrywają ponadto istotną rolę w patogenezie zwiększonej kurczliwości naczyń. Produkowane są przez komórki śródbłonka łożyska naczyniowego, komórki mięśni gładkich oraz fibroblasty. Destrukcyjny wpływ reaktywnych form tlenu ma duże znaczenie w patogenezie wielu chorób, takich jak: miażdżyca, niedokrwienie, niewydolność serca, niewydolność naczyń mózgowych oraz niewydolność oddechowa.
MATERIAŁ I METODY: W grupie badanej było 36 pacjentów (31 kobiet i 5 mężczyzn; średnia wieku 47 ± 7 lat) z chorobą tkanki łącznej (23 osoby z toczniem rumieniowatym układowym oraz 13 osób z twardziną układową), w grupie kontrolnej 40 zdrowych ochotników (37 kobiet i 3 mężczyzn; średnia wieku 50 ± 2 lata). W pracy oceniono całkowitą zdolność antyoksydacyjną w surowicy, wykorzystując metodę redukcji rodnika DPPH i rodnika $ABTS^{\bullet+}$.
WYNIKI: U pacjentów z chorobą układową tkanki łącznej aktywność antyoksydacyjna osocza jest mniejsza w porównaniu z grupą zdrowych ochotników.
WNIOSKI: W pracy wykazano różnice w aktywności antyoksydacyjnej między zdrowymi ochotnikami a pacjentami z chorobą tkanki łącznej. Parametry te mogą być monitorowane podczas terapii przez modulowanie metabolizmu oksydacyjnego i stymulację aktywności antyoksydantów.

SŁOWA KLUCZOWE
stres oksydacyjny, choroby tkanki łącznej, dysmutaza ponadtlenkowa

INTRODUCTION

In recent years, interest in the imbalance between the production of reactive oxygen species and the efficiency of antioxidant systems in homeostasis disorders has increased [1]. Oxidative stress and inflammation are hypothesized to play important roles in a wide variety of diseases, such as cardiovascular diseases, diabetes, cancer, and autoimmune diseases [2].
Systemic lupus erythematosus is a chronic inflammatory autoimmune disease with multifactorial aetiology and a variety of clinical manifestations. Most often, the disease manifests itself in musculoskeletal, dermal, mucosal, renal and cytopenia symptoms [3].
The mechanism of autoantibody production in diseases such as systemic lupus erythematosus has not yet been identified. It is possible that the consequent production of autoantibodies may be the result of reactive oxygen species (ROS) attack on DNA, causing changes in the structure at the macromolecular level. It is therefore postulated that in chronic inflammatory diseases, ROS generated by phagocytic cells may cause damage to DNA and autoantibodies are produced to a self-antigen [4].
Scleroderma or systemic sclerosis (SSc) is an acquired, generalized, autoimmune disorder characterized by microvascular dysfunction and excessive collagen deposition in the skin and several viscera. Vascular abnormalities are a hallmark of the disease, including vascular hyperreactivity and obliterative changes in small vessels [5]. An immune-inflammatory injury in the microvascular system occurs, leading to dysregulation of vascular tone control.
There is consistent evidence that oxidative stress – a shift in the balance between ROS generation and an efficient system of antioxidants in favor of ROS production – is associated with SSc. However, its specific involvement in the pathogenesis of vascular injury and fibrosis is still unclear [6]. A considerable number of studies show increased oxidative processes in SSc patients. Many researchers indicate that ROS, which cause increased degradation of nitric oxide and defects in endothelial function, play an essential role in the pathogenesis of increased vascular contractility. Reactive oxygen species are produced in the vascular bed by the endothelial cells, smooth muscle cells and fibroblasts. The destructive effect of ROS is of great importance to the pathogenesis of many diseases such as atheromatosis, ischemia, heart failure, cerebral insufficiency and respiratory failure [7,8,9].
The study was approved by the local Ethics Committee and all the patients and control subjects gave a written consent.

MATERIAL AND METHODS

Patient groups

A total of 36 patients – 31 females, 5 males (mean age 47 ± 7 years) with connective tissue diseases (lupus – 23, scleroderma – 13) and 40 healthy volunteers – 37 females, 3 males (mean age 50 ± 2 years) of both

sexes were enrolled in the study. Patients who were pregnant or current smokers, or patients diagnosed with diabetes, hypercholesterolemia, or a severe disease (e.g. cardiac or hepatic failure, cancer, or gangrene), or patients with active infections were excluded from the study. Patients who had not been stable in their current treatment for at least 3 months were also excluded.

Material

The blood samples were taken from the antecubital vein and anticoagulated with heparin. Then plasma was obtained by centrifugation of the blood.
2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammononium salt (ABTS), 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) and hydrogen peroxide were purchased from Sigma Chemical Co.

DPPH radical scavenging activity

1.76 ml (phosphate buffered saline) PBS was added to 0.04 ml of blood plasma. Then the sample was mixed with 0.2 ml 1.0 mM DPPH in methanol. After 30 min the optical density of the sample was measured at 532 nm. Next we calculated the antioxidant activities with the following formula:

DPPH inhibition (%) =
= [1-(sample OD/control OD)] x 100

The antioxidant activity was expressed as a percentage of DPPH inhibition [10,11,12].

ABTS radical scavenging activity

0.005 ml of blood plasma was added to 0.095 ml of phosphate buffered saline (PBS). Next the sample was mixed with 0.02 ml of 10 mM hydrogen peroxide and incubated at 37°C for 5 min. Then 0.03 ml of 1.25 mM ABTS in PBS and 0.03 ml of 1 unit/ml peroxidase in PBS were added to the mixture. The sample was incubated at 37°C for 10 min and the optical density of the sample was measured at 416 nm [11]. Afterwards the antioxidant activities were calculated with the following formula:

$ABTS^{\bullet+}$ inhibition (%) =
= [1-(sample OD/control OD)] x 100

RESULTS

The following figures (Fig. 1, 2) illustrate the study results.

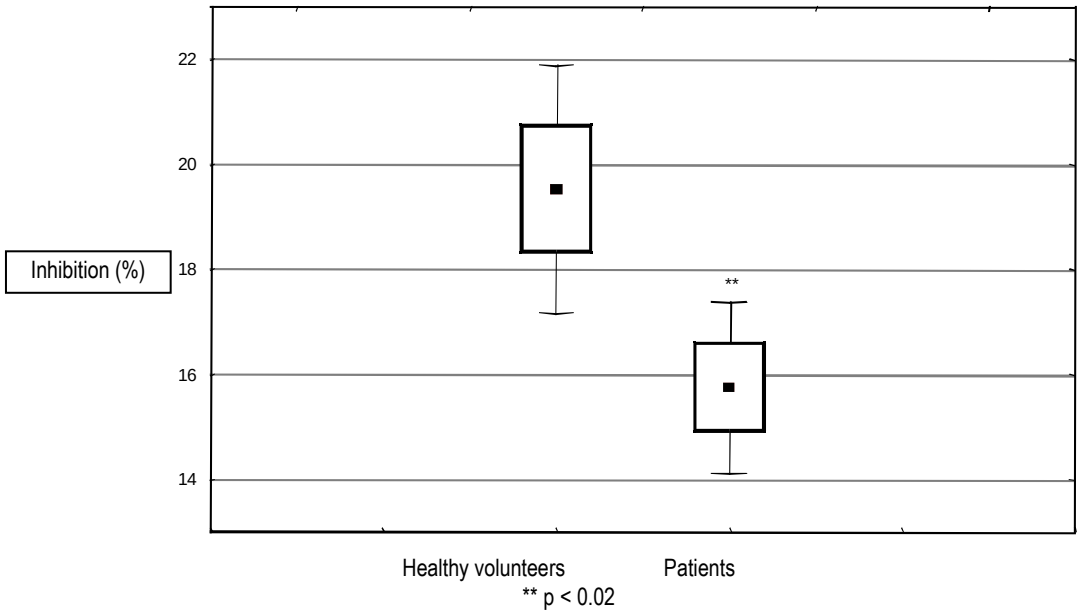

Fig. 1. DPPH inhibition (%).
Ryc. 1. DPPH inhibicja (%).

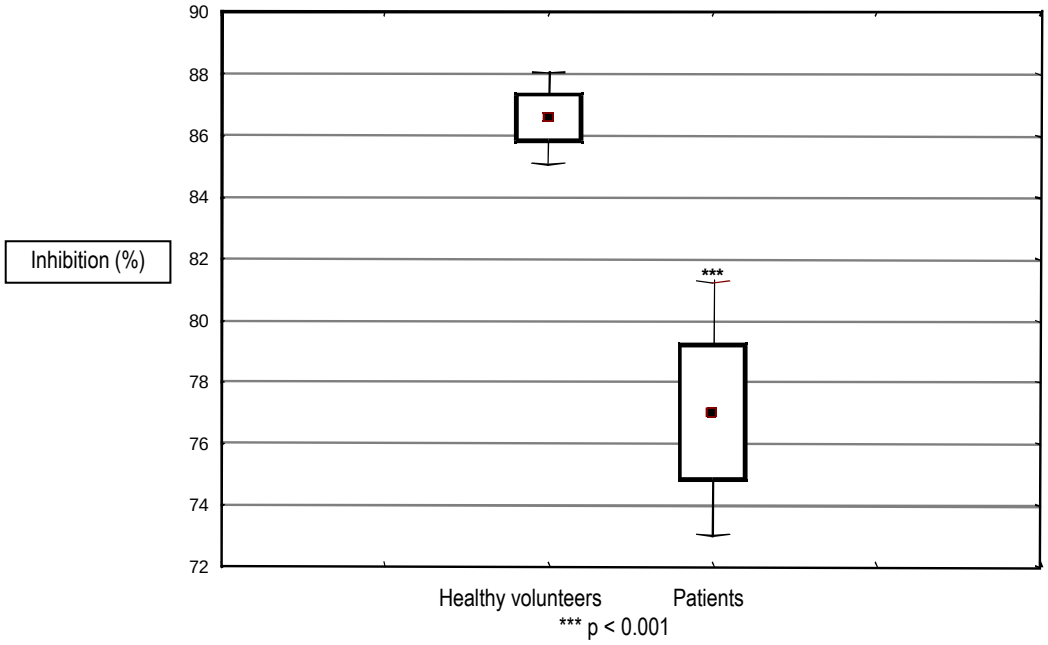

Fig. 2. ABTS$^{\bullet+}$ inhibition (%).
Ryc. 2. ABTS$^{\bullet+}$ inhibicja (%).

The blood plasma of the patients with connective tissue diseases had lower antioxidant activities as compared to the blood plasma of the healthy volunteers. To perform statistical calculations, the STATISTICA 12 program was used.

DISCUSSION

Various methods are used in different laboratories to evaluate the total antioxidant capacity of blood plasma. Oxidative stress is considered to be a major pathogenic agent of most degenerative diseases. Antioxidants inhibit or delay the oxidation process by blocking the initiation or propagation of oxidizing chain reactions that may cause some chronic diseases, including cardiovascular diseases or connective tissue diseases. The ABTS method is suitable for monitoring lipophilic antioxidants. The scavenging activity of the DPPH free radical is a common method to evaluate antioxidant activity. Oxidative stress biomarkers may play a role in future to assist the clinical decision to administer antioxidant therapy. Therapeutic interventions that replenish the redox balance or decrease exposure to ROS and augment antioxidant defenses might be beneficial adjunctive therapy in the treatment of oxidative damage in systemic lupus erythematosus [13]. Oxidative stress is one of the important factors that contribute to tissue damage in SSc. Since the physiological response to oxidative stress is regulated by multiple antioxidant systems, it is important to measure the total antioxidant capacity in biological specimens quantitatively [14]. The results of the total antioxidant capacity in healthy subjects performed by different methods and the same method in different centers differ, but it is possible to determine the direction of total antioxidant capacity changes in pathological states. Total antioxidant capacity is also reduced in such diseases as Graves' disease, phenylketonuria, acute myocardial infarction, diabetes mellitus, and lung cancer [15].

CONCLUSIONS

This study shows the differences in the antioxidant activity in healthy volunteers and patients with connective tissue diseases. These parameters can be monitored during treatment by modulating oxidative metabolism and by stimulating antioxidant activity [16].
The blood plasma of the patients had lower antioxidant activities compared to the blood plasma of the healthy controls. Furthermore, we are of the opinion that there is a great need for further research in the field of antioxidant activity.

Author's contribution

Study design – B. Przywara-Chowaniec, S. Seget
Data collection – M. Dróżdż, A. Puzio
Data interpretation – Z. Czuba, E. Nowalany-Kozielska
Statistical analysis – M. Gawlikowski
Manuscript preparation – B. Przywara-Chowaniec, S. Seget
Literature research – M. Dróżdż, A. Puzio

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