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ANTIOXIDANT CAPACITY OF SERUM MEASURED USING TEMPO SCAVENGING ASSAY IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Serum antioxidant capacity was measured using TEMPO radical scavenging assay. Radical concentration was determined by electron paramagnetic resonance (EPR) spectroscopy. The dependence on disease activity, gender and smoking habits in patients with rheumatoid arthritis (RA) was investigated. The decrease in serum antioxidant capacity was observed for moderate and severe activity of RA as compared with control group. There was no relation observed between serum antioxidant capacity and gender, or between serum antioxidant capacity and smoking habits.

INTRODUCTION

Oxidative stress can be a causative factor in several diseases (Chauhan, Chauhan and Brown, 2009; Denisov and Afanas'ev, 2005), including autoimmune ones such as rheumatoid arthritis (RA). It occurs when the balance between prooxidants and antioxidants is disturbed in favour of the former, and can be a consequence of decreased antioxidants level (Halliwell and Gutteridge, 2007). Therefore, the determination of antioxidant capacity of biological system could potentially give valuable information about the mechanism and probability of autoimmune diseases (Halliwel and Gutteridge, 1990).

There are different methods of evaluating the antioxidant capacity of human organism, some of them being based on a partial neutralization of a stable radical, like DPPH (2,2-diphenyl-1-picrylhydrazyl) (Martinez, Valek, Rešetić and Ferenec Ružić, 2006) and nitroxide radicals (Fuchs, Groth, Herrling and Zimmer, 1997), by body fluids such as blood plasma or serum.

The antioxidant capacity of human serum measured with TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) radical is due mainly to vitamin C level (Piehl, Facorro, Huarte, Desimone, Copello, Díaz and Rubín de Celis, 2005), which is one of two (the other being uric acid) most abundant water-soluble low molecular weight antioxidants in human blood. Nonetheless, the reaction of TEMPO with other antioxidants, like anthocyanins, is also possible (Butković, 2009).

The aim of this work was to establish the relationship between antioxidant capacity of serum measured with TEMPO radical assay and DAS28 (Disease Activity Score) factor. DAS28 was chosen as a measure of the activity of rheumatoid arthritis, for patients with moderate and very active disease as compared with healthy subjects. Additionally, the influence of smoking habits and gender on the antioxidant capacity of serum was considered.

EXPERIMENTAL DETAILS

Patients

Studies were performed on 98 patients diagnosed with RA and 22 healthy subjects (control group). Fasting blood samples were collected in the morning and serum samples were stored at -80°C.

TEMPO scavenging by human serum

The procedure was based on that described by Piehl et al. (Piehl *et al.*, 2005). 20 μl of TEMPO solution (6.4 mM in PBS (phosphate buffered saline), pH = 7.4) was added to 480 μl of human serum. EPR spectra of TEMPO were recorded at room temperature for 30 minutes. Nitroxide signal intensity was measured as the total height of the low field peak.

The antioxidant capacity of serum was calculated as the vitamin C equivalent concentration using the calibration curve of TEMPO concentration change after 30 minutes (Δc) *vs* vitamin C concentration.

EPR measurements were performed on a X-band Miniscope MS200 spectrometer (Magnettech, Berlin, Germany) using the following instrument settings: modulation amplitude, 0.1 mT; centre field, 334 mT; sweep width, 8 mT; sweep time, 20 s; microwave power, 13 mW.

DAS28

Factor DAS28 was calculated according to the equation:

$DAS28 = 0.56 \cdot (TEN)^{1/2} + 0.28 \cdot (SW)^{1/2} + 0.7 \cdot LN(OB) + 0.014 \cdot VAS$

where: TEN - number of joints tender to the touch, SW - number of swollen joints, ESR - erythrocyte sedimentation rate, VAS - patient assessment of disease activity [mm].

Statistical analysis

Data were analyzed with the Mann–Whitney U test and the Kruskal–Wallis one-way analysis of variance by ranks**.** Statistical analysis was performed using data analysis software system Statistica 9 (StatSoft Inc.).

RESULTS AND DISCUSSION

Rheumatoid arthritis severity

Antioxidant capacity of serum for patients with severe, moderate and inactive or absent RA was compared. Statistically significant ($p<0.001$) decrease was observed for both moderate (n = 25) and severe (n = 68) RA groups as compared with control group (n = 24) [Fig. 1]. Mean value of Vitamin C equivalent for control group is over two times greater than for the group of patients with severe RA and about 30% than for the moderate RA group. This suggests a relation between oxidative stress and this autoimmune disease severity.

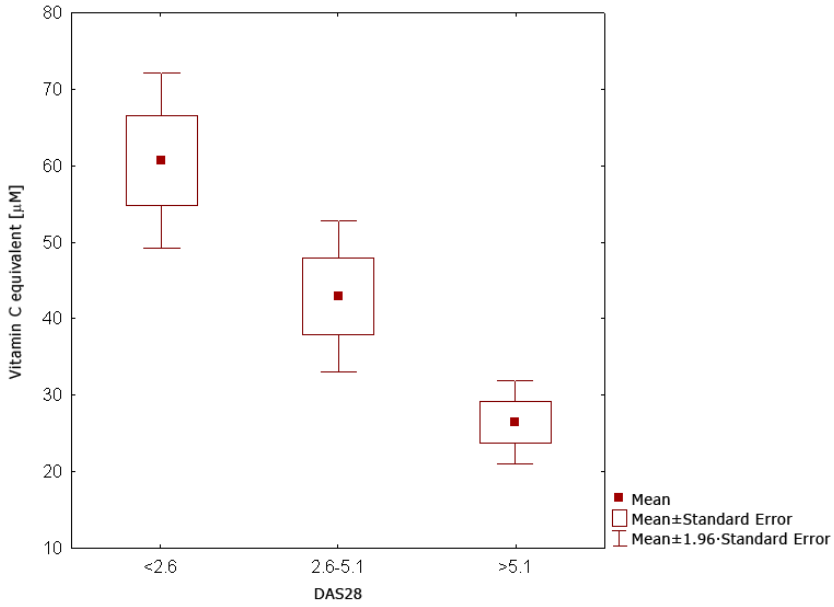

Fig. 1. Antioxidant capacity dependence on severity of RA. DAS28 values < 2.6 correspond to the absence or remission of RA, those between 2.6 and 5.1 to moderate activity of RA, and those > 5.1 to severe activity.

Gender

The potential influence of gender was investigated. However, the results did not show any relation between gender and antioxidant capacity of serum in RA diagnosed group [Table 1].

Table 1. Antioxidant capacity for women and men

Group	n	Vitamin C equiv. Mean ± SE [µM]
Women	18	32.9 ± 5.8
Men	80	32.9 ± 3.2

Smoking habits

Among patients, there was no statistically significant difference between smokers and non-smokers groups [Table 2], irrespectively of the RA severity. This is in opposition to the frequently observed decrease of antioxidant capacity in healthy smokers (Rahman, Swarska, Henry, Stolk and MacNee, 2000). However, the potential effect of smoking during RA exacerbation

may be masked by a strong decrease of serum antioxidant capacity due to RA itself.

Table 2. Antioxidant capacity for smoking and non-smoking patients

Group	n	Vitamin C equiv. Mean ± SE [μM]
Smokers	17	31.8 ± 3.0
Non-smokers	79	39.4 ± 7.8

CONCLUSIONS

The antioxidant capacity of human serum measured with TEMPO assay is decreased in patients with rheumatoid arthritis as compared with a control group of healthy volunteers. This effect is more pronounced for severe AR activity group than for moderate AR activity one.

There was no significant influence of gender or smoking habits on antioxidant capacity in patients diagnosed with RA.

TEMPO radical scavenging assay utilizing EPR spectroscopy can be used as an optional method for evaluation of oxidative stress in various diseases.

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