Clinical and Experimental Rheumatology 2007; 25: 112-114

# Persistence of protein oxidation products and plasma antioxidants in juvenile idiopathic arthritis. A one-year follow-up study

J. Renke[1], A. Szlagatys[1], R. Hansdorfer-Korzon[2], M. Szumera[1], B. Kamińska[1], N. Knap[3], S. Popadiuk[1], A. Szarszewski[1], M.Woźniak[3]

*[1]Department of Pediatrics, Pediatric Gastroenterology and Oncology, Medical University of Gdańsk, ul. Nowe Ogrody 1-6, 80-803 Gdańsk, Poland; [2]Department of Rehabilitation and [3]Department of Medical Chemistry, Medical University of Gdańsk, ul. Dębinki 1, 80-211 Gdańsk, Poland.*

*Joanna Renke, MD, PhD; Agnieszka Szlagatys, MD, PhD; Rita Hansdorfer-Korzon, MD, PhD; Małgorzata Szumera, MD, PhD; Barbara Kaminska, MD, PhD; Narcyz Knap, MD, PhD; Stefan Popadiuk, MD, PhD; Adam Szarszewski, MD, PhD; Michał Woźniak, MD, PhD, Professor.*

*Please address correspondence to: Prof. Michal Woźniak, MD, PhD, Department of Medical Chemistry, Medical University of Gdańsk, ul. Dębinki 1, 80-211 Gdańsk, Poland.*
*E-mail: mwozniak@amg.gda.pl*

*Received on March 1, 2005; accepted in revised form on September 28, 2006.*



**Key words:** protein oxidation, antioxidants, juvenile idiopathic arthritis.

*Abbreviations:*

| | |
|---|---|
| GPx: | glutathione peroxidase |
| Hb: | hemoglobin |
| JIA: | juvenile idiopathic arthritis |
| NSAID: | nonsteroid anti-inflammatory drug |
| ROS: | reactive oxygen species |
| SOD: | superoxide dismutase |
| TAS: | total antioxidant status |

## ABSTRACT

**Objective.** *Plasma protein oxidation products and blood antioxidants, like superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status (TAS) were investigated in children with juvenile idiopathic arthritis (JIA) in a year follow-up study.*

**Methods.** *Carbonyl group content within plasma proteins, activity of red blood cell SOD and GPx, as well as the blood TAS level were determined in 14 children with JIA twice, namely at the admission to the hospital (Time 0 = T0) and then after a year of treatment (Time 1 = T1).*

**Results.** *An increased level of plasma protein carbonyls was observed in both assessments (T0 and T1) as compared to control. However there was no significant difference in plasma carbonyls level between the initial (T0) and final (T1) examination of the patients. Similarly, SOD activity was higher in children with JIA as compared to control subjects and did not change significantly after a year of follow-up. Red blood cell GPx activity remained within the normal range throughout the study. Interestingly, the blood TAS level was initially comparable to control and rose significantly after the year of treatment.*

**Conclusions.** *A level of plasma protein oxidation products remains significantly higher in children with JIA as compared to healthy subjects. The lack of accumulation of plasma protein carbonyls may result from efficient proteolysis in childhood and/or adaptive increase of the blood TAS level in the course of effective anti-inflammatory therapy. Analysis of plasma oxidative stress markers and antioxidant potential of the blood might be helpful in monitoring the clinical treatment of children suffering from JIA.*

## Introduction

Juvenile idiopathic arthritis is a chronic inflammatory disease of unknown etiology. There is evidence however of reactive oxygen species (ROS) involvement in the inflammatory process of JIA (1, 2). Oxidative stress is suggested to damage joints by causing degradation of hyaluronic acid, proteoglycans and collagen (3). An ischemia-reperfusion injury mechanism is also believed to be of importance in JIA patients (4).

Protein oxidative modification seems to be a good marker of oxidative stress (5, 6). A significant number of studies have shown the accumulation of protein oxidation products during lifespan (reviewed by Levine, 2002). The aim of the study was to find out if the protein oxidation products are accumulated in children with JIA in the course of the disease. Moreover, plasma antioxidants were assayed to find a possible correlation with the level of oxidized proteins.

## Material and methods

The clinical study was carried out at the Department of Pediatrics and the Department of Medical Chemistry, Medical University of Gdansk in the years of 1996 -1999. There were 14 children (6 males and 8 females) aged from 5 to 17 years (mean 11.5 ± 3.5) with juvenile idiopathic arthritis (JIA) and a control group of 30 healthy subjects (14 males and 16 females) aged from 6 to 18 years (mean 12.5 ± 2.5) enrolled in the study. An informed consent was signed by all the children participating in the study or/and their parents. The study project was approved of by the Local Research Ethics Committee.

The patients were diagnosed according to the criteria of the International League of Associations for Rheumatology, Durban 1997 (7). They all suffered from polyarticular JIA of moderate activity. The patients were treated continuously with oral nonsteroid anti-inflammatory drugs (NSAID) and periodically with low doses of oral corticosteroids during exacerbations (22.8 – 45.6 mg/kg of prednisone – equivalents per year). No patient was administered corticosteroids within the last month of the follow-up.

The level of plasma protein carbonyl groups, enzymatic activities of the red blood cell SOD and GPx, as well as the blood TAS level were assayed twice, namely at the admission to the hospital (Time 0 = T0) and then after a year of treatment (Time 1 = T1).

The carbonyl group content within plasma proteins was determined by

**Table I.** Biochemical analyses of the blood taken twice in a group of children with JIA, namely at the admission to the hospital (T0) and after a year of follow-up (T1), as compared to control group of healthy subjects (HS). Non-significant p values denoted as p = NS.

| Patients | JIA at Time 0 (T0) | JIA at Time 1 (T1) | Healthy subjects (HS) |
|---|---|---|---|
| Carbonyls [nmol/mg of protein] | 1.69 ± 0.63<br>n = 14<br>p < 0.001 vs. HS | 1.64 ± 0.52<br>n = 14<br>p < 0.001 vs. HS | 0.87 ± 0.18<br>n = 30 |
| SOD [IU/g of Hb] | 1982.9 ± 883.7<br>n = 14<br>p < 0.05 vs. HS | 2061.1 ± 1482.8<br>n = 13<br>p < 0.05 vs. HS | 1203.2 ± 395.6<br>n = 30 |
| GPx [IU/g of Hb] | 19.4 ± 16.5<br>n = 13<br>p = NS vs. HS | 32.1 ± 23.1<br>n = 14<br>p = NS vs. HS | 22.6 ± 12.6<br>n = 30 |
| TAS [mmol/L] | 1.68 ± 0.22<br>n = 14<br>p = NS vs. HS | 1.97 ± 0.34<br>n = 14<br>p < 0.05 vs. HS | 1.59 ± 0.32<br>n = 30 |

Carbonyls: protein oxidation products; SOD: superoxide dismutase; GPx: glutathione peroxidase; TAS: total antioxidant status.

the method of Levine *et al*. (1990) (8), based on the reaction of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH). The results were expressed in nmol per mg of protein.

Enzymatic activity of SOD and GPx in the red blood cells as well as TAS of the blood were assayed with RANDOX kit, namely Ransod according to Minami and Yoshikawa method (1979), Ransel according to Paglia and Valentine method (1967) (9) and TAS according to Rice-Evans and Miller method (1994) (10) respectively. The activities of SOD and GPx were expressed in International Units per gram of hemoglobin. TAS was expressed in mmol per liter.

The results were presented as mean values with standard deviation calculated. The means were compared using Student t-test. The correlation analysis was performed by Pearson's correlation test.

## Results

The results of plasma protein carbonyls content are presented in Table I. The levels of protein carbonyls in both assays (T0 and T1) were not statistically different, however they were significantly higher than those in healthy controls ($p < 0.001$).

The levels of antioxidants are presented in Table I. Enzymatic activities of SOD in the red blood cells of children with JIA in both assays (T0 and T1) were not statistically different, however they were significantly higher than those of healthy controls ($p < 0.05$). Enzymatic activities of the red blood cell GPx remained within the reference range throughout the study in both JIA and control subjects.

The blood level of TAS taken at the admission of JIA patients to the hospital (T0) did not statistically differ from that of healthy subjects. However it increased significantly after a year of medical treatment ($p < 0.05$).

No correlation was found between the plasma protein carbonyls content and the activity of plasma antioxidants in children with JIA.

## Discussion

Many reports have suggested a possible role of reactive oxygen species in juvenile idiopathic arthritis (1, 11). In the studies an increased level of plasma protein oxidation products was found in children with JIA. The results are consistent with the previous study of ours (2). Lipid peroxidation products seemed increased (12) also in adults with rheumatoid arthritis. Moreover, some studies reported increased protein oxidation as well as lipid peroxidation products in the synovial tissue and articular fluid from patients with rheumatoid arthritis (13, 14).

The group of JIA patients was relatively homogenous in respect to clinical assessment and the course of the disease which allowed to objectively evaluate the levels of plasma protein oxidation products in children with JIA and obtain comparable results within a year of the follow-up study. To date, there has been no report available on a long-term analysis of protein oxidation products in the plasma of patients suffering from a chronic inflammatory disease. The plasma protein carbonyls were found to be significantly increased in children with JIA as compared to healthy controls, both at the admission (T0) and after a year of medical treatment (T1). A reason for sustained elevated plasma carbonyls in children with JIA still needs to be elucidated. On one hand it might be assumed that the treatment had not been aggressive enough to effectively reduce the level of oxidative stress associated with the pathogenesis and progression of the inflammatory process. On the other hand, however, it might have been the proper therapy that prevented a potential accumulation of the protein oxidation products in plasma in the course of the disease. The latter reflection is in concordance with the values of the blood TAS taken in JIA children which became significantly higher after a year of anti-inflammatory therapy. Although neither plasma protein carbonyls nor TAS are routine means to evaluate an oxidative stress-dependent inflammatory process, they seem clinically applicable as sensitive markers for monitoring and fine-tuning medical therapy in case of a chronic inflammatory disease. It is noteworthy that plasma protein carbonyls level is a very dynamic marker of ROS-dependent oxidative stress intensity.

A possible accumulation of protein oxidation products is the subject of many studies. It has been well established that they do accumulate during life span (6, 15, 16). Increase of plasma and tissue protein carbonyls is an age-related process (17, 18). A mechanism of the phenomenon remains unknown yet (6). Some studies support a hypothesis that ROS-dependent oxidative stress increases with age (19). Simultaneously, decreased activity of proteases and increased levels of endogenic proteolysis inhibitors are observed (15, 20, 21)

which may result in lower degradation of oxidized proteins. Thus no accumulation of protein carbonyls observed in the patients with JIA may be partly due to effective proteolysis in childhood.

In both examinations the activity of superoxide dismutase in the red blood cells was higher in patients with JIA as compared to healthy controls. The higher activity of antioxidative enzyme seems to be a response to increasing generation of reactive oxygen species during inflammatory process. The increased activity of SOD in children with JIA was also reported in other studies (2).

The analysis of antioxidant status in children with JIA showed significant increase of TAS level after one year of the disease duration. All children were treated with the anti-inflammatory drugs in the period between the two examinations. The increase of antioxidative capacity of the blood is probably due to the treatment resulting in the improvement of the primary and secondary antioxidants efficacy, which may be an initial phase of restoring the antioxidative-prooxidative balance of the organism. The finding of increased TAS level after a year of treatment seems useful for monitoring therapy.

The presented results of our study are preliminary. The studied group is limited due to very strict clinical criteria for enrollment of subjects into the study. Further research is necessary to obtain more in-depth conclusions on the accumulation of protein oxidation products and the antioxidant status in the course of a chronic inflammatory process.

## Conclusions

Analysis of oxidative stress intensity markers may be helpful in monitoring the medical treatment of children with JIA. The lack of accumulation of protein oxidation products in children with JIA may be a result of effective proteolysis in childhood and/or the treatment. An increased level of TAS after one year of the disease duration seems due to efficient anti-inflammatory therapy. The finding of increased TAS level may be of clinical importance for monitoring the efficacy of JIA therapy.

## References


1. SKLODOWSKA M, GROMADZINSKA J, BIERNACKA M *et al*.: Vitamin E, thiobarbituric acid reactive substance concentrations and superoxide dismutase activity in the blood of children with juvenile rheumatoid arthritis. *Clin Exp Rheumatol* 1996; 14: 433-9.
2. RENKE J, POPADIUK S, KORZON M, BUGAJCZYK B, WOZNIAK M: Protein carbonyl groups' content as a useful clinical marker of antioxidant barrier impairment in plasma of children with juvenile chronic arthritis. *Free Radic Biol Med* 2000; 29: 101-4.
3. BURKHARDT H, SCHWINGEL M, MENNINGER H, MACARTNEY HW, TSCHESCHE HL: Oxygen radicals as effectors of cartilage destruction. Direct degradative effect on matrix components and indirect action via activation of latent collagenase from polymorphonuclear leukocytes. *Arthritis Rheum* 1986; 29: 379-87.
4. MERRY P, WINYARD PG, MORRIS CJ, GROOTVELD M, BLAKE DR: Oxygen free radicals, inflammation, and synovitis: the current status. *Ann Rheum Dis* 1989; 48: 864-70.
5. CHEVION M, BERENSHTEIN E, STADTMAN ER: Human studies related to protein oxidation: protein carbonyl content as a marker of damage. *Free Radic Res*. 2000; 33 (Suppl.): S99-108.
6. LEVINE RL: Carbonyl modified proteins in cellular regulation, aging, and disease. *Free Radic Biol Med* 2002; 32: 790-6.
7. PETTY RE, SOUTHWOOD TR, BAUM J *et al*.: Revision of the proposed classification criteria for juvenile idiopathic arthritis: Durban, 1997. *J Rheumatol* 1998; 25: 1991-4.
8. LEVINE RL, GARLAND D, OLIVER CN *et al*.: Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol* 1990; 186: 464-78.
9. PAGLIA DE, VALENTINE WN: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70: 158-69.
10. RICE-EVANS C, MILLER NJ: Total antioxidant status in plasma and body fluids. *Methods Enzymol* 1994; 234: 279-93.
11. ARAUJO V, ARNAL C, BORONAT M, RUIZ E, DOMINGUEZ C: Oxidant-antioxidant imbalance in blood of children with juvenile rheumatoid arthritis. *Biofactors* 1998; 8: 155-9.
12. TAYSI S, POLAT F, GUL M, SARI RA, BAKAN E: Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis. *Rheumatol Int* 2002; 21: 200-4.
13. GRIGOLO B, ROSETI L, FIORINI M, FACCHINI A: Enhanced lipid peroxidation in synoviocytes from patients with osteoarthritis. *J Rheumatol* 2003; 30: 345-7.
14. LUNEC J, HALLORAN SP, WHITE AG, DORMANDY TL: Free-radical oxidation (peroxidation) products in serum and synovial fluid in rheumatoid arthritis. *J Rheumatol* 1981; 8: 233-45.
15. BEAL MF: Oxidatively modified proteins in aging and disease. *Free Radic Biol Med* 2002; 32: 797-803.
16. STADTMAN ER, LEVINE RL: Protein oxidation. *Ann N Y Acad Sci* 2000; 899: 191-208.
17. MUTLU-TURKOGLU U, ILHAN E, OZTEZCAN S, KURU A, AYKAC-TOKER G, UYSAL M: Age-related increases in plasma malondialdehyde and protein carbonyl levels and lymphocyte DNA damage in elderly subjects. *Clin Biochem* 2003; 36: 397-400.
18. OLIVER CN, AHN BW, MOERMAN EJ, GOLDSTEIN S, STADTMAN ER: Age-related changes in oxidized proteins. *Biol Chem* 1987; 262: 5488-91.
19. SOHAL RS, WEINDRUCH R: Oxidative stress, caloric restriction, and aging. *Science* 1996; 273: 59-63.
20. GRUNE T, MERKER K, SANDIG G, DAVIES KJ: Selective degradation of oxidatively modified protein substrates by the proteasome. *Biochem Biophys Res Commun* 2003; 305: 709-18.
21. SZWEDA PA, FRIGUET B, SZWEDA LI: Proteolysis, free radicals, and aging. *Free Radic Biol Med* 2002; 33: 29-36.