THE JURNAL OF PRVENTIVE MEDICINE
2001; 9(2): 38-42

ANTIOXIDANT ENZYME LEVELS IN REACTIVE ARTHRITIS AND RHEUMATOID POLYARTHRITIS

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Abstract. This study is based on the comparative analysis of 38 patients with reactive arthritis defined by Amor criteria, a group of 33 patients with rheumatoid polyarthritis and 50 healthy controls. Antioxidant systems (glutathione peroxidase, superoxide dismutase, catalase) were investigated in inflammation consecutive to immune and infectious syndromes. Our study showed that reactive arthritis and rheumatoid arthritis is associated with enhanced oxidative stress. The lower levels of natural blood antioxidant enzyme, were related to severe forms of disease. The investigation of oxidative metabolism lies at the basis of antioxidant therapy that could influence the natural course of reactive arthritis and polyarthritis.
Key words: reactive arthritis, rheumatoid poyarthritis, oxidative stress, oxygen free radicals,
catalase, glutathione peroxidase, superoxide dismutase

Rezumat. Acest studiu se bazează pe analiza comparativă a 38 de pacienţi cu artrită reactivă definită prin criteriul Amor, un grup de 33 pacienţi cu poliartrită reactivă şi 50 de voluntari sănătoşi. Au fost investigate sistemele antioxidante (glutation peroxidaza, superoxid dismutaza, catalaza) care apar în urma inflamaţiei consecutive sindroamelor imuno- infecţioase. Studiul nostru demonstrează că artrita reactiva si poliartrita reumatoida sunt asociate cu creşterea stress-ului oxidativ. Nivelul scăzut al enzimelor antioxidante din sînge a fost prezent la cazurile cu forme severe ale bolii. Investigarea metabolismului oxidativ stă la baza terapiei cu antioxidanţi naturali care ar putea influenţa evoluţia normală a artritelor reactive şi a poliartritelor.
Cuvinte cheie : artrita reactiva, poliartrita reumatoida, stress oxidativ, radicali liberi ai oxigenului, catalaza, glutation peroxidaza, superoxid dismutaza

INTRODUCTION

Reactive arthritis is defined as an aseptic arthritis induced by an extraarticular infectious agent. A comprehensive definition of reactive arthritis includes the following features (1):

- It refers to inflammatory arthropathies developing elsewhere in the body and long before the triggering infectious episode.
- The disease is systemic and, despite its name, involves more than the joints.
- The entry of the infectious agent is more often urogenital, digestive and respiratory.
- A predisposing genetic background

exists.

In 1986, Amor (2) included reactive arthritis in the group of seronegative spondyloarthopathies.

The pathogenesis of reactive arhritis is complex given the predispozing genetic factors, the multitude of involved germs with various entry routes, and the likelihood of some still unidentified factors.

The mechanism of arthritis consists in the presence and persistence in the joints of bacterial antigens (such as lipopolysaccharides and proteoglycans) that can mimic the tissue antigens of the host, thus triggering the immune tolerance (3). The complex and partially elucidated inflammatory process in reactive arthritis, was recently investigated by the study of oxidative metabolism. Free radicals may play a role in the causation and preservation of inflammation in reactive arthritis, considered as inflammation mediators. Oxygen free radicals are rapidly and effectively neutralized by a series of enzymatic and non-enzymatic antioxidant systems (4). In inflammatory rheumatic diseases (rheumatoid polyarthritis and reactive arthritis), oxygen free radicals and antioxidant systems stimulate the immune response (5,6).

Catalase is prevalent in erythrocytes and liver.It contains four hemin groups per molecule. It is found in the cell in small bodies as peroxisomes which also contain the enzymes (oxidases) which form hydrogen peroxide, such as amino acid oxidases and xanthin oxidase.The enzyme catalyses the same reaction catalysed by peroxidase, which is known as its peroxidase activity. In addition, it can catalyse a specific reaction in which the substrate (hydrogen donor) is hydrogen peroxide. It removes two hydrogen atoms from one molecule of hydrogen peroxide (the substrate), liberating oxygen, and gives them to another molecule of hydrogen peroxide, forming water.This is known as its catalase activity (7).

Superoxide dismutase (SOD) removes the toxic superoxide radical (O_2^-) formed by the partial reduction of oxygen in tissues. The superoxide dismutase found in the cytosol contains Cu^{2+} and Zn^{2+}, while that found in the mitochondria contains Mn^{2+}. The function of SOD seems to be that of protecting aerobic organisms against the potential deleterious effects of superoxide anion (O_2) (7).

Glutathione peroxidase (GSHPx) containing the trace selenium, provides the second line of defence against hydroperoxides before they can damage membranes and other cell components (4,7).

Studies on the comparative levels of antioxidant systems in rheumatoid polyarthritis and reactive polyarthritis demonstrate the role of peroxidation process in the pathogenesis of these disorders (8,9,10,11).

MATERIAL AND METHOD

The study included three groups:

GROUP A: 38 patients with reactive arthritis (17 of them with Reiter syndrome) diagnosed according to the criteria suggested by Amor (2); there were 22 men and 16 women aged between 17 and 64 years (mean age 32.0 years).

GROUP B: 33 patients (5 men and 28 women) aged between 20 and 70

years (mean age 47.5 years) with polyarthritis diagnosed according to ARA criteria (2) (1987) as Steinbrocker stage I - 18.2%, II - 45.4%, III - 27.2%, IV - 9.0%. Waaler-Rose reaction was positive in 75.8% of the cases.

GROUP C (controls): 50 blood donors recorded in Iasi Transfusion Center.

The following biochemical tests were assessed in both groups in comparison with controls: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSHPx).

CAT was investigated according to the method described by Aebi (12).The method is based on estimation of hydrogen peroxide decomposed during 30 seconds at 25^0C. Enzymatic activity was expressed K/g of hemoglobin (Hb). K is the reaction rate constant defined by Aebi (12).

The erythrocyte antioxidant system SOD was investigated according to the method described by Minami and Yoshikawa (13). The method is based on the inhibition by SOD of pirogallol-induced nitroblue tetrazolium (NBT) reduction.

Plasma GSHPx activity was investigated according to the method described by Fukuzawa (14).

The statistical processing of data was made using EPI INFO 6 programme and involved means, standard deviations ,"t" test, coefficients of variation (CV%) and confidence intervals (CI 95%).

RESULTS AND DISCUSSION

Erythrocyte CAT activity was significantly increased in the patients with reactive arthritis compared with controls (group C).This increase may be due to some oxidative damages challanged by oxigen free radicals increased levels in these pathological condition (Table 1).

Table 1. Mean values of catalase activity

	Reactive arthritis A	Rheumatoid polyarthritis B	Voluntary blood donors C
No. cases	25	33	50
Catalase K/g Hb.	680.33 ± 123.20***	550.25 ± 99.64*	577.55 ± 104.59
95% CI	(629.57 - 731.09)	(516.25 - 584.25)	(548.56 - 606.54)
% CV	18.11	18.11	18.11

* $p< 0.05$ *** $p< 0.001$

The mean values (table 2) of erythrocyte SOD activity in reactive arthritis patients was significantly decreased compared with only control C, the patients group of B having lower values. Decreasing of erythrocyte SOD in the patients with rheumatoid arthritis represents a laboratory evidence of inflammatory syndrome. More evidence become available suggesting oxygen

free radicals such superoxide anion, hydroxyl radical (OH.), and related oxygen species such as hydrogen peroxide (H_2O_2), and singlet oxygen (O^-) ,are involved in the pathogenesis of rheumatoid arthritis. During phagocytosis granulocytes and macrophages produce large amounts of O_2^- and H_2O_2 (4). Oxygen free radicals destroy lipids by a process called lipid peroxidation. In reactive arthritis patients, SOD, an enzyme destroying O_2^-, given systemically or locally, induces a decrease of inflammation (15).

Table 2. Mean values of erythrocyte SOD activity

	Reactive arthritis A	Rheumatoid polyarthritis B	Voluntary blood donors C
No. cases	38	33	50
SOD (U/Ht x 100)	6.6 ± 1.3*	6.045 ± 1.355***	7.19 ± 0.71
95% CI	(6.19 – 7.01)	(5.59 – 6.51)	(6.99 – 7.39)
% CV	19.70	22.48	9.87

* $p< 0.05$ *** $p< 0.001$

Table 3. Mean values of glutathione peroxidase activity

	Reactive arthritis A	Rheumatoid polyarthritis B	Voluntary blood donors C
No. cases	25	33	50
GSHPx (U/l)	89.25 ± 28.05	81.45 ± 21.15	85,00 ± 14,20
95% CI	(77.69 – 100.81)	(74.23 – 88.67)	(81.06 – 88.94)
% CV	31.43	25.60	16.70

A slight but unsignificant increase of GSHPx values was found in the patients with reactive arthritis compared with controls (group C) and the polyarthritis patients (Table 3).

CONCLUSIONS

1. The results showed significant increase of CAT, as the first antioxidant defence line against peroxidation damages in reactive arthritis patients compared with voluntary blood donors.
2. The SOD activity was significantly decreased in both pathological conditions compared with the voluntary blood donors.
3. The GSHPx activity showed unsignificant modifications in rheumatoid arthritis and polyarthritis patients in comparison with the voluntary blood donors.
4. The results confirm the important role of oxidative stress in the pathogenesis of reactive arthritis and rheumatoid polyarthritis, suggesting that in both diseases

antioxidant treatment is required.

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