Int J Biol Med Res. 2013; 4(2) : 3088-3090

BioMedSciDirect Publications

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International Journal of Biological & Medical Research

Journal homepage: www.biomedscidirect.com

Original article

Oxidative stress and antioxidant status in patients with rheumatoid arthritis

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ARTICLE INFO

Keywords:
Glutathione peroxidase
Malondialdehyde
Rheumatoid arthritis
Superoxide dismutase.

ABSTRACT

Rheumatoid Arthritis (RA) is a multifactorial autoimmune disease affecting around 1% of the world's population leading to autoimmune arthritis. Though the exact etiology of RA remains unknown, various environmental and biological triggers have been suspected. The most important factor which is implicated in the pathogenesis of RA is oxidative stress. However the exact relationship between antioxidants and lipid peroxidation is not yet known in patients of RA. Hence the case control study was conducted in 50 patients of rheumatoid arthritis (Group II) diagnosed by criteria's recommended by American Rheumatology Association were compared with age and sex matched 50 normal healthy controls (GroupI). Malondialdehyde (MDA) a marker of oxidative stress, antioxidant enzymes Glutathione peroxidase (GPx) and Superoxide dismutase (SOD) were measured by enzymatic spectrophotometric method. MDA levels was significantly increased ($p< 0.01$) in RA patients as compared to controls. The concentrations of GPx and SOD were significantly lower in RA patients as compared to normal healthy adults. Our findings indicate that oxidative stress does occur in patients RA patients. Treatment with antioxidant therapy to limit the disease process of RA patients needs to be assessed.

1. Introduction

Rheumatoid arthritis (RA) is the most common inflammatory arthritis affecting about 0.8% of general populations [1]. RA is characterized by persistent inflammation in the synovial membranes of joints, associated with migration of activated phagocytes and other leukocytes into synovial and periarticular tissue [2].

About 70% of patients with RA have progressive disease, which follows a chronic pattern with periods of exacerbation and remission. A further 25% of patients have intermittent disease which is characterized by brief attacks of inflammation with intermittent remissions in which there is little or no disease activity. The remaining 5% of patients have a malignant form of disease with extra-articular manifestations such as vasculitis. The disease can begin at any age, but it most often starts after age 40 and before 60. In some families, multiple members can be affected, suggesting a genetic basis for the disorder. The cause of rheumatoid arthritis is unknown. Even though infectious agents such as viruses, bacteria, and fungi have long been suspected, none has been proven as the cause. The cause of rheumatoid arthritis is a very active area of worldwide research. Some scientists believe that the tendency to develop rheumatoid arthritis may be genetically inherited. It is suspected that certain infections or factors in the environment might trigger the immune system to attack the body's own tissues, resulting in inflammation in various organs of the body such as the lungs or eyes. Regardless of the exact trigger, the Reactive oxygen species (ROS) has been implicated to gear up immune system to promote inflammation in the joints and occasionally other tissues of the body.

Reactive oxygen species (ROS) play an important role in tissue injury in this disease [3]. The most important characteristic of toxic free radicals either in vivo or in vitro is peroxidation of lipids resulting in tissue damage and death of affected cells [4] and the prime targets of ROS attack are the polyunsaturated fatty acids

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(PUFA) in the membrane lipids causing lipid peroxidation which may lead to disorganization of cell structure and function. The harmful effect of reactive oxygen species is neutralized by a broad class of protective agents termed antioxidants which prevents oxidative damage by reacting with free radicals before any other molecules can become a target and antioxidant enzymes super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) play an important role in the protection of cells and tissues against free radical mediated tissue damage [5,6]

Numerous studies have indicated discordant results in patients with RA. Hence, the aim of the present study was to assess the lipid peroxidation and effect of enzymatic antioxidants in patients with RA.

2. MATERIALS AND METHODS

Present study was designed as a case control study conducted in the Department of Biochemistry, NKP Salve Institute of Medical Sciences. 50 patients of RA and 50 normal healthy controls matched for age, sex were included in this study. Patients were diagnosed by the criteria's recommended by the American Rheumatism Association [7]. Patients suffering from diabetes mellitus, abnormal renal and hepatic function, history of myocardial infarction patients on long term medications, other causes of arthritis like osteoarthritis, gout were excluded as these diseases directly or indirectly lead to free radical production.

The research protocol was approved by ethics committee of the institution. 5 ml of venous blood samples was collected in EDTA bottles and plain bulbs from patients with rheumatoid arthritis and normal healthy individuals. Blood samples was centrifuged at 3000g for 10 minutes.

Malondialdehyde was measured by the method of randox laboratory.This method was based on the fact that lipid peroxide condense with 1 methyl -2 phenyl indole (MPI) under acidic conditions resulting in the formation of a red chromophore. To determine specifically lipid peroxide in plasma, proteins are precipitated to remove water soluble MPI reactive substance. The level of lipid peroxide is expressed in term of malondialdehyde. Tetramethoxypropane, which is converted quantitatively to MDA was used as standard.

The erythrocytic GPx was estimated by spectrophotometeric kit method [8], the priniciple being that GPx catalyses the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH the oxidized glutathione is immediately converted to reduced form with a concomitant oxidation of NADPH to NADP. The decrease in absorbance was measured at 340nm.

SOD was measured from hemolysate by spectrophotometeric enzymatic kit method [9]. The principle employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye.The statistical analysis was done by student t- test.

3. Results & Discussion

Table 1 shows comparison of MDA, SOD, GPx levels in RA with control subjects. MDA levels were found to be significantly elevated in patients with RA compared to control. F. Karatas etal [10], S. D. Walwadkar etal [11], Cimen MY etal [12],Desai PB etal [13], Sarban S etal [14], Shivani Jaswal etal [15], Kamanli A etal [16] reported higher MDA levels in patients with RA in their studies [10-16]. It indicates increased oxidative stress because of increased lipid peroxidation. MDA, the product of lipid per oxidation can exacerbate inflammation by producing immunogenic molecule and causing cell damage. The polyunsaturated fatty acids are more prone for lipid peroxidation and loss of lubrication of synovial fluid is very important consequence of exposure of synovial fluid to superoxide and hydrogen peroxide. Our results differed from other studies which found that MDA levels are high when patients are in active stage of the disease and our patients were not in the active stage [17-18]. Oleveri et al [19] reported no change in lipid peroxidation in RA patients but Saowanee et al [20] and Ozkan etal [21] observed increased MDA levels but not statistically significant to conclude that there was an increased oxidative stress in RA patients because supporting results were not obtained from total oxidative status and SOD measurement. SOD, an antioxidant enzyme, catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Superoxide anion, which is believed to be one of the initiators of free radical production reactions plays an important role in the determination of the levels of antioxidant enzyme SOD. At physiological rates of hydrogen peroxide generation, the glutathione system is important in catabolising hydrogen peroxide. GPx reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water.

Decreased activities of antioxidant enzymes (SOD & GPx) were found to be statistically significant in RA patients compared to controls in our study. The results of present study are similar to that observed by F. Karatas etal [10] , N Aryaeian etal [22], Bae SC etal[23] , Desai PB etal[13] . The decreased antioxidant enzymes levels might be due to utilization of these enzymes for detoxification of free radicals.

Our findings are contradictory to the findings of Akyol etal [24] & Ozkan etal [21] who reported no change in SOD levels of RA patients & controls.

However, Cimen MY etal[12] observed higher SOD levels and unchanged GPx activity in RA patients suggesting that excessive free radical production may be through xanthine – xanthine oxidase system rather than an impaired antioxidant system and therapeutic use of XO enzyme inhibitors can be beneficial. Similarly D.Vijay kumar etal [25] reported increased levels of SOD and GPx in their study which could be due to dismutate the excess superoxide radicals that are generated and diffused from the inflammatory sites and due to over expression of antioxidant defence system of RA patients.

Thus in RA patients overproduction of free radicals lead to imbalance in the oxidant antioxidant system. This imbalance might play a role in the tissue damage and inflammation process in this disease.

Hence with regards to this, further studies regarding the administration of antioxidant therapy along with conventional drugs and safety and efficacy of such therapy in limiting the disease process needs to be carried out.

Table I. Levels of MDA and Enzymatic Antioxidants in healthy subjects and in patients with RA

Group I (n=50)		Group II (n=50)
MDA (nmol/litre)	1.09+ 0.26	3.48+0.50*
SOD (U/ml)	357.63+ 82.24	200.07+ 25.71*
GPx (IU/L)	10188.36+ 843.62	4692.37+ 463.74*

4. References

[1] Harris JRE, Budd R, Firestein G, Genovese M, Sergent J, Uddy S, Sledge G (2005).Nutrition and Rheumatic Diseases In:Textbook of Rheumatology. Eds, Kelley.volume 1, 7ST ed: Elsevier & Saunders Inc Philadelphia, pp.: 833-73.2.

[2] Mulherin D, Fitzgerald O, Bresnihan B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum. 1996;39 : 115-24.

[3] Mapp PI, Grootveld MC, Blake DR. Hypoxia , oxidative stress and rheumatoid arthritis. Br Med Bull. 1995; Apr; 51(2):419-36.

[4] Catalá A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chem Phys. Lipids. 2009; 157:1–11.

[5] Halliwell B. Free radicals, antioxidants and human disease: curiosity, cause, or consequence? Lancet 1994; 344: 721-724.

[6] Ray, G. and Husain, S.H. Oxidants, antioxidants and carcinogenesis. Indian J. Exp. Biol. 2002; 40: 1213-1232.

[7] Arnett FC, Edworthy SM, Bloch DA etal. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31:315-323.

[8] Paglia,D.E.and Valentine W.N.J.Lab.Clin.Med.1967;70:158.

[9] Woolliams JA, Wiener G, Anderson PH, McMurray CH. Research in veterinary science 1983;34:253-256.

[10] F. Karatas, I. Ozates, H. Canatan , I. Halifeoglu , M. Karatepe & R. Colak. Antioxidant status & lipid peroxidation in patients with rheumatoid arthritis. Indian J Med Res .October 2003; 118: pp 178-181

[11] S. D. Walwadkar, A.N. Suryakar, R.V. Katkam, K.M. Kumbar and R.D. Ankush. Oxidative stress and calcium-phosphorus levels in rheumatoid arthritis. Indian Journal of Clinical Biochemistry, 2006 ; 21 (2): 134-137.

[12] Cimen MY, Cimen OB, Kacmaz M, Ozturk HS, Yorgancioglu R, Durak I. Oxidant / antioxidant status of the erythrocytes from patients with rheumatoid arthritis. Clin Rheumatol. 2000; 19(4):275-7.

[13] Desai PB, Manjunath S, Kadi S, Chetana K, Vanishree J. Oxidative stress and enzymatic antioxidant status in rheumatoid arthritis: a case control study. Eur Rev Med Pharmacol Sci. Nov 2010;14(11):959-67.

[14] Sarban S, Kocyigit A, Yazar M, Isikan UE. Plasma total antioxidant capacity, lipid peroxidation, and erythrocyte antioxidant enzyme activities in patients with rheumatoid arthritis and osteoarthritis.Clin Biochem. 2005 Nov; 38 (11): 981-6.

[15] Shivani Jaswal , Harish Chander Mehta, Arun Kumar Sood and Jasbinder Kaur. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. Clinica Chimica Acta., December 2003; Volume 338, Issues 1-2: Pages 123-129.

[16] Kamanli A, Naziroglu M, Aydilek N, Hacievliyagil C. Plasma Lipid peroxidation and antioxidant levels in patients with rheumatoid arthritis. Cell Biochem Funct. 2004; 22(1):53-7.

[17] Ozgunes H, Gurer H, Tuncer S. Correlation between plasma malondialdehyde and ceruloplasmin activity values in rheumatoid arthritis. Clin Biochem. 1995; 28: 193-4.

[18] Gambhir J K, Lali P, Jain AK. Correlation between blood antioxidant levels and lipid peroxidation in rheumatoid arthritis. Clin Biochem. 1997; 30: 351-5.

[19] Olivieri O, Girelli D, Trevisan MT, Bassi A, Zorzan P, Bambara LM, et al. Red blood cell susceptibility to lipid peroxidation, membrane lipid composition and antioxidant enzymes in patientswith rheumatoid arthritis. J Rheumatol 1991; 18 : 1263-4.

[20] Saowanee Kajanachumpol, Monchand Vanichapuntu1, Oravan Verasertniyom , Kitti Totemchokchyakarn and Mongkol Vatanasuk. Levels of plasma lipid peroxide products and antioxidant status in rheumatoid arthritis. Southeast Asian J Trop Med Public Health. June 2000; Vol 31 No. 2;336 .

[21] Ozkan Y, Yardym-Akaydyn S, Sepici A, Keskin E, Sepici V, Simsek B. Oxidative status in rheumatoid arthritis. Clin Rheumatol. 2007 Jan; 26(1):64-8.

[22] N Aryaeian , M Djalali , F Shahram , A Djazayeri , MR Eshragian, N Nadery, F Fatehi ,M Zarei, M Chamari. The Effect of Conjugated Linoleic Acids, Vitamin E and Their Combination on Lipid Peroxidation in Active Rheumatoid Arthritis. Iranian J Publ Health. 2009; Vol. 38, No.2: 79-89.

[23] Bae SC, Kim SJ, Sung MK. Inadequate antioxidant nutrient intake and altered plasma antioxidant status of rheumatoid arthritis patients. J Am Coll Nutr. 2003 Aug; 22(4):311-5.

[24] Akyol O, Isci N, Temel I, Ozgocmen S, Uz E. The relationships between plasma and erythrocyte antioxidant enzymes and lipid peroxidation in patients with rheumatoid arthritis. Joint Bone Spine 2001; 68 : 311-7.

[25] D. Vijayakumar, K. Suresh and S. Manoharan. Lipid peroxidation and antioxidant status in blood of rheumatoid arthritis patients. Indian Journal of Clinical Biochemistry, 2006, 21 (1) 104-108