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Oxidative Stress in Patients with Rheumatoid Arthritis

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ABSTRACT

Background: Rheumatoid arthritis is an autoimmune disease of unknown etiology, characterized by articular inflammation. Oxidative damage induced by reactive oxygen species has been related to the pathophysiology of rheumatoid arthritis in several studies, although results have been inconsistent and contradictory. **Objective:** To determine oxidative stress markers in patients with rheumatoid arthritis. **Methods:** Descriptive cross-sectional study in rheumatoid arthritis patients and healthy controls. In peripheral blood samples from all study subjects, lipid peroxide (thiobarbituric acid reactive substances) and protein carbonyl levels were quantified as oxidative damage markers; superoxide dismutase and glutathione peroxidase activities, glutathione concentration, and the reduced glutathione/oxidized glutathione ratio were analyzed as antioxidant defense indicators. Mann-Whitney *U* tests were run. Statistical significance (α) was 0.05%. **Results:** We included 29 rheumatoid arthritis patients, 10 with active disease, and 41 healthy controls. Age was higher in the rheumatoid arthritis group; there were no differences in female:male ratio between groups. Oxidative damage was higher in rheumatoid arthritis patients; however, there was no difference between patients with active or inactive rheumatoid arthritis. Antioxidant enzyme activities, glutathione concentration, and reduced glutathione/oxidized glutathione ratio were higher in rheumatoid arthritis patients than in controls. **Conclusions:** Antioxidant levels were higher in rheumatoid arthritis patients than in healthy controls; however, they were insufficient to prevent oxidative damage. This suggests an active oxidative process in rheumatoid arthritis patients. (REV INVEST CLIN. 2015;67:46-53)
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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disease of unknown etiology, characterized by symmetric inflammation of both small and large joints, with possible systemic damage[1]. Rheumatoid arthritis affects mostly the economically productive age group; its prevalence increases with age and is approximately 25-times higher in women than in men[2]. Rheumatoid arthritis is suspected based on

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clinical criteria and there are no specific markers. Such criteria, however, are mainly used for classification and not for diagnosis. Therefore, the population with clinical criteria for RA is highly heterogeneous and markers are needed to distinguish between groups. Rheumatoid arthritis, despite being a disease predominantly of the joints, can have different clinical patterns with severe extra-articular involvement; it is considered a systemic disease with an insidious course, response to treatment, and outcome[3]. Long-term studies suggest that in most patients, RA is a progressive disease, with severe articular damage as seen by radiographic methods, deterioration of physical function, and significant increase in mortality[4].

Aerobic production of the energy needed for the various cell processes occurs via oxidative phosphorylation, in which redox reactions in the mitochondria lead to synthesis of adenosine triphosphate. During this process, oxygen serves as the final acceptor of electrons from cytochrome oxidase *c*, yielding water as the final product. Some of the intermediate products of this tetravalent reduction are partially reduced oxygen metabolites, which are highly reactive and are termed reactive oxygen species (ROS). ROS participate in multiple physiological processes, are part of the defense system against microorganisms[5], and are involved in the intracellular signaling pathways[6]. Approximately 2% of the partially reduced oxygen escapes the mitochondria and is capable of damaging molecules and cell structures, particularly proteins, lipids, DNA, membranes and structural elements of the extracellular matrix such as proteoglycans and collagen[7,8]. Among ROS, superoxide radical ($O_2^{\bullet -}$), hydroxyl radical (HO•) and peroxynitrite ($ONOO^-$) are highly reactive[9]. The balance between ROS production and elimination, either by antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), or by non-enzymatic molecules, such as glutathione (GSH) and vitamins A, C, and E, contributes to maintain homeostasis in aerobic organisms. Loss of this balance leads to oxidative damage and is known as oxidative stress[10].

Oxidative stress is involved in the pathophysiology of several diseases, RA among them[11]. Despite this, there is no consensus on how to determine oxidative stress in a uniform and consistent way that can be used in clinical decision-making. For the rheumatologist that is not directly involved in basic research and works within the clinical context, the approach to the physical chemistry of the cell may be difficult. Different practical forms of assessing oxidative stress have been proposed. Among them, the reduced glutathione/oxidized glutathione (GSH/GSSG) ratio[12-14], the ratio between activities of SOD, GPx and CAT (SOD/GPx + Cat)[15], and determination of the redox potential of GSH[16].

Research on the role of oxidative stress in the initiation and perpetuation of the inflammatory process in RA has produced inconsistent and contradictory results. Structural damage mediated by ROS has been reported[17]; however, the oxidative damage increases[18] or does not change[19]. Similarly, reports of the non-enzymatic and enzymatic antioxidant activities in RA patients show divergent results. The relationship between oxidative stress and RA is still uncertain. Therefore, the aim of this study was to determine, in RA patients as compared to healthy controls, the oxidative damage, quantified as lipid peroxidation and protein carbonyl levels, activity of the antioxidant enzymes SOD and GPx, and GSH/GSSH ratio, its correlation with other biochemical markers, and the correlation of the disease with clinical, biochemical, and oxidative stress indicators.

METHODS

Study population and samples

A cross-sectional study was done at the Outpatient Clinic (Unidad de Atención Médica Ambulatoria) of the Mexican Social Security (Instituto Mexicano del Seguro Social, IMSS) at La Paz, Baja California Sur, Mexico. The study was approved by the local research committee. Written informed consent was obtained prior to inclusion. Patients with a diagnosis of RA according to the American College of Rheumatology criteria[20] were selected from the outpatient clinic of the Rheumatology Service. Patients with diabetes mellitus, extreme obesity (body mass index, BMI > 40), hypertension state II or higher, neoplasia, smoking history, active infection, or recent (< 1 year) surgery were excluded[21]. The control group consisted of healthy volunteer donors of the blood bank at the IMSS Regional Hospital (Hospital de Zona más Medicina Familiar 1).

Patients were divided into two groups based on the inflammatory activity of the disease, by using the RA activity index (DAS28)[22], which assesses the number

of painful joints, number of swollen joints, erythrocyte sedimentation rate, and global evaluation of the disease by the patient, with a cutoff point of ≥ 2.9 for active inflammatory process. Sociodemographic and clinical data of patients were obtained from the medical charts. To eliminate potential confounding factors, subjects that had the following conditions in the month prior to sampling were excluded: antioxidant supplement intake, treatment against dyslipidemia, recent infections, liver or kidney disease, neoplasm, stroke, acute myocardial infarction, or accidental trauma[23]. From RA patients and controls included in the study, a peripheral blood sample was collected in a heparinized container (Vacutainer®), which was kept at 4°C prior to transport to the Oxidative Stress Laboratory (Laboratorio de Estrés Oxidativo) at the Centro de Investigaciones Biológicas del Noroeste, S.C. (CIBNOR) for analysis.

Laboratory procedures

In all blood samples, activity of the antioxidant enzymes SOD and GPx, GSH and GSSG concentrations, and levels of lipid peroxidation (thiobarbituric acid reactive substances, TBARS) and protein carbonyls were determined and GSH/GSSG and SOD/GPx ratios were calculated. SOD activity was determined by spectrophotometry, using the method based on the xanthine/xanthine oxidase system as constant generator of $O_2\bullet^-$ which, upon contact with nitro blue tetrazolium, reduces it to formazan; SOD, catalyzes the dismutation of $O_2\bullet^-$ into hydrogen peroxide (H_2O_2) and inhibits the reduction of nitro blue tetrazolium[24]; data are shown in international units of SOD activity per gram of hemoglobin (Hb). One unit of SOD activity is defined as the amount of enzyme that inhibits the maximum reduction of nitro blue tetrazolium by 50%. GPx activity was analyzed by monitoring the continuous decrease in reduced nicotinamide adenine dinucleotide phosphate (NADPH) concentration while maintaining constant GSH levels[25]. Data are shown in international units of GPx activity per gram of Hb; one unit of GPx activity is defined as the amount of enzyme that oxidizes 1 µmol NADPH per minute. The GSH concentration was quantified by following the change in absorbance at 412 nm generated when GSH reacts with 5,'5-ditiobis-(2-nitrobenzoic) acid (DTNB, Ellman's reagent); GSSG concentration was assessed by the first derivatization of GSH with 2 vinylpyridine[26]. To obtain the GSH and GSSG concentrations in the samples, results were compared with those from a standard curve (0-0.4 µM for GSSG and 0-0.8 µM for GSH); data are shown in nmol per gram of Hb. Oxidative damage to lipids was assessed by quantifying TBARS. The method is based on the reaction of lipid hydroperoxides and aldehydes formed from peroxidation reactions with thiobarbituric acid to produce malondialdehyde, a crystalline pink pigment with maximum absorption at 532-535 nm[27]; data are shown as nmol per gram of Hb. Oxidative damage to proteins was analyzed by the quantification of carbonyl groups in proteins by the derivatization in the presence of 2,4-dinitrophenylhydrazine, yielding the stable product dinitrophenylhydrazone, which can be detected by spectrophotometry at 370 nm[28]; data are presented in nmol per gram of Hb.

Statistical analysis

Descriptive statistics were used for the sociodemographic variables; inferential statistics were used to probe for differences between groups (control vs. RA; active vs. inactive RA). To test for differences between medians, Mann-Whitney *U* tests were performed and 25th and 75th quartiles were calculated as a measure of dispersion. Bivariate correlation between the variables was analyzed by Spearman's correlation index (rho). Multiple linear regression analysis was used to assess the participation of the independent variables, age, BMI, Hb concentration, TBARS levels, protein carbonyl concentration, SOD and GPx activities, GSH and GSSG concentrations relative to the DAS28 index (dependent variable). The regression models were constructed using the "stepwise" analysis, with the aim of selecting the predicting variables that significantly maximize the model's r^2 (coefficient of determination)[29]. To assess the homoscedasticity and fit, as well as to detect atypical observations in the data, an analysis of the residuals distribution was performed. The significance level α was set at ≤ 0.05. All statistical analyses were run using SPSS v.20.0.

RESULTS

Blood samples were collected from 29 RA patients, 10 of whom had active disease; the control group consisted of 41 healthy blood donors. The median age in the RA group was 48 years (25th and 75th percentiles, 43.5-70.0) and of the control group, 38 years (33.5-44.5) ($p < 0.05$); the female:male ratio was 9:1 in the RA group and 8.4:1 in the control group (Table 1).

Table 1. Clinical characterisitics of rheumatoid arthritis patients and controls

	RA patients (n = 29) median (25th–75th percentiles)		Controls (n = 41) median (25th–75th percentiles)
	Active disease (n = 10)	Inactive disease (n = 19)	
Age, years	48.0 (43.5-70.0)	48.5 (31.2-54.2)	38.0†# (33.5-44.5)
Sex (F:M)	9:1	8.6:1.0	8.4:1.0
RA duration, years	7.0 (3-15)	2.0 (1.0-13.5)	NA
DAS28	4.3 (4.0-5.2)	2.1* (1.8-2.3)	NA
Hb	10.7 (7.4-13.6)	14.6* (12.7-15.7)	15.4# (13.8-17.1)
BMI	25.0 (23.5-27.2)	26.1 (25.2-31.2)	26.0 (24.6-31)

RA: rheumatoid arthritis; DAS28: disease activity index; Hb: hemoglobin concentration, g/dl; BMI: body mass index; NA: not applicable. Statistical significance by Mann-Whitney *U* test $p < 0.05$.
*patients with inactive disease vs. patients with active disease;
†controls vs. patients with inactive disease;
#controls vs. patients with active disease.

When compared with controls, the RA patients had higher oxidative damage to proteins ($p = 0.02$) and lipids ($p < 0.01$) (Table 2). No significant differences were found in the oxidative damage markers between patients with active and those with inactive disease ($p > 0.05$) (Table 3). The RA patients had higher antioxidant enzyme activities and higher GSH concentration than controls (Table 2). The GSH/GSSG ratio was significantly higher in RA patients than in controls ($p < 0.01$) (Table 2), with the same ratios between patients with active or inactive disease. The SOD/GPx ratio was significantly lower in RA patients than in the control group ($p < 0.01$) (Table 2). However, there were no significant differences in the SOD/GPx ratio between patients with active or inactive disease ($p = 0.15$) (Table 3).

Results from the correlation analysis are summarized in table 4. In RA patients, the GSH/GSSG ratio was positively related to the GSH concentration ($r = 0.57$; $p < 0.01$). The GSH and GSSG concentrations were positively related to each other ($r = 0.74$; $p < 0.01$). The SOD activity was negatively related to the protein carbonyl content ($r = –0.46$; $p = 0.01$), and was positively related to the TBARS levels ($r = 0.63$; $p < 0.01$) and the GSSG concentration ($r = 0.79$; $p < 0.01$). The GPx activity was positively correlated to the TBARS levels ($r = 0.52$; $p < 0.01$) and GSSG concentration ($r = 0.72$; $p < 0.01$).

Multiple linear regression models were generated to assess the effect of the covariates age, BMI, TBARS

Table 2. Oxidative stress indicators in blood samples from rheumatoid arthritis patients and controls

	Patients (n = 29) median (25th–75th percentile)	Controls (n = 41) median (25th–75th percentile)	p
TBARS	0.08 (0.07-0.11)	0.07 (0.06-0.08)	< 0.01
Protein carbonyls	15.4 (7.2-21.8)	9.8 (5.87-129)	0.02
SOD	269.1 (167.1-484.9)	191.7 (118.4-296.4)	0.02
GPx	49.5 (42.6-61.2)	4.6 (2.22-8.37)	< 0.01
GSH	36.3 (15.9-66.4)	23.1 (10.9-35.1)	0.01
GSSG	3.5 (2.05-5.9)	4.2 (2.4-6.4)	0.54
GSH/GSSG	10.2 (7.8-132.8)	5.4 (4.8-230.3)	< 0.01
SOD/GPx	4.7 (3.5-6.6)	34.7 (21.8-93.1)	< 0.01

TBARS: thiobarbituric acid reactive substances levels (nmol/g Hb); protein carbonyls concentration (nmol/g Hb); SOD: superoxide dismutase activity (U/g Hb); GPx: glutathione peroxidase activity (U/g Hb); GSH: reduced glutathione concentration (nmol/g Hb); GSSG: oxidized glutathione concentration (nmol/g Hb); p = statistical significance by Mann-Whitney *U* test.

Table 3. Clinical characteristics and oxidative stress indicators in blood from rheumatoid arthritis patients with active compared to inactive disease

	Active disease (n = 10) median (25^{th}–75^{th} percentile)	Inactive disease (n = 19) median (25^{th}–75^{th} percentile)	p
Age, years	48.0 (43.5-70)	48.5 (31.2-54.2)	0.27
Duration of illness, years	7.0 (3-15)	2.0 (1-13.5)	0.06
TBARS	0.08 (0.06-0.11)	0.09 (0.07-0.12)	0.43
Protein carbonyls	18.7 (7.3-25.8)	10.8 (6.7-17.9)	0.19
SOD	227.6 (163.7-320.9)	304.0 (164.3-513.6)	0.46
GPx	49.4 (40.7-58.3)	54.3 (42.45-75.7)	0.51
GSH	21.9 (13.3-44.1)	47.2 (17.2-91.4)	0.08
GSSG	3.3 (2.5-5.5)	3.6 (1.8-6.2)	0.77
GSH/GSSG	6.6 (5.3-7.9)	13.0 (9.2-14.6)	0.00
SOD/GPx	4.3 (3.2-4.7)	5.8 (3.7-6.4)	0.15

Active disease: DAS28 ≥ 2.9; TBARS: thiobarbituric acid reactive substances levels (nmol/g Hb); protein carbonyls concentration (nmol/g Hb); SOD: superoxide dismutase activity (U/g Hb); GPx: glutathione peroxidase activity (U/g Hb); GSH: reduced glutathione concentration (nmol/g Hb); GSSG: oxidized glutathione concentration (nmol/g Hb); p = statistical significance by Mann-Whitney *U* test.

levels, protein carbonyl content, and activities of SOD and GPx on the DAS28 index. Table 5 shows the final model for the correlation of BMI, Hb concentration, GPx activity, and GSSG concentration with the DAS28 index in RA patients ($r^2 = 0.481$; $p < 0.01$). The constant variance and the normal distribution of the residuals did not suggest any trend, confirming the sufficiency of the adjusted model (Fig. 1).

DISCUSSION

Despite the divergent opinions on the role of oxidative stress in the genesis and perpetuation of damage observed in RA, there is evidence that it may participate in the pathogenesis of the disease[30]. Oxidative damage in synovial fluid, with structural changes in hyaluronic acid, cartilage, and collagen, as well as increased lipid peroxidation, protein carbonyl content, and DNA alterations, have been reported[2].

In this study, higher oxidative damage, as assessed by TBARS and protein carbonyl levels, was found in RA patients compared to healthy controls. The GSH concentration, GPx activity, and the activity of SOD were also higher in RA patients than in healthy individuals; however, the GSH/GSSG ratio was higher and the SOD/GPx ratio was lower in RA patients than in controls. There were no significant differences in the markers of

Table 4. Bivariate correlation between oxidative stress indicators in blood from rheumatoid arthritis patients

	GSH	TBARS	SOD	GSSG
GSH/GSSG				
– Rho	0.57			
– p	< 0.01			
GPx				
– Rho	0.44	0.52	0.66	0.72
– p	0.02	< 0.01	< 0.01	< 0.01
GSH				
– Rho			0.51	0.74
– p			< 0.01	< 0.01
TBARS				
– Rho			0.63	0.54
– p			< 0.01	< 0.01
CP				
– Rho			–0.46	
– p			0.01	
SOD				
– Rho				0.79
– p				< 0.01

TBARS: thiobarbituric acid reactive substances levels (nmol/g Hb); protein carbonyls concentration (nmol/g Hb); SOD: superoxide dismutase activity (U/g Hb); GPx: glutathione peroxidase activity (U/g Hb); GSH: reduced glutathione concentration (nmol/g Hb); GSSG: oxidized glutathione concentration (nmol/g Hb); rho: Spearman's coefficient; p = statistical significance.

Table 5. Lineal multivariate model adjusted for the index of disease activity (DAS28) in rheumatoid arthritis patients

Model	Variable	Non-standardized coefficient						
		b	Standard error	t	p	r^2	F	*p**
DAS28	Intercept	6.406	1.449	4.421	< 0.01	0.481	5.564	<0.01
	BMI	−0.110	0.040	−2.732	< 0.01			
	Hb	−0.017	0.067	−2.535	< 0.01			
	GPx	0.042	0.013	3.116	< 0.01			
	GSSG	−0.141	0.064	−2.184	0.04			

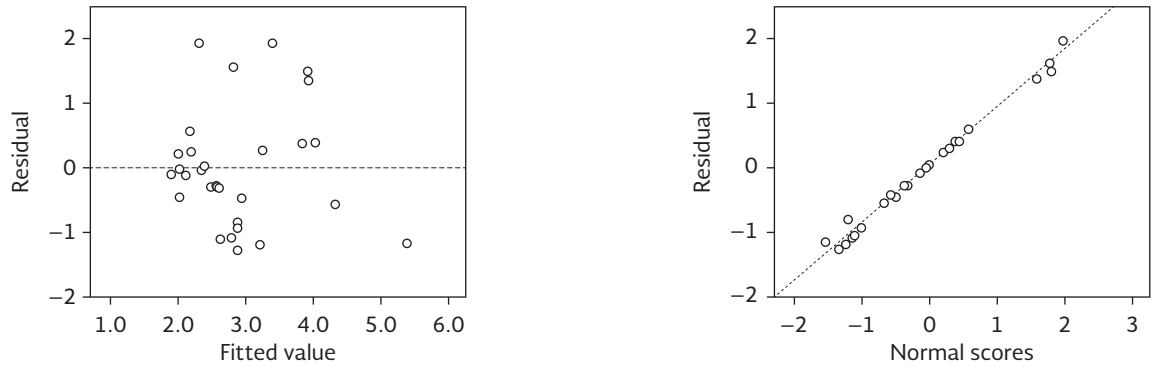
DAS28: index of disease activity; BMI: body mass index; Hb: hemoglobin concentration (g/dl); GPx: glutathione peroxidase activity (U/g Hb); GSSG: oxidized glutathione concentration (nmol/g Hb).

oxidative stress quantified in this study between patients with active compared to inactive RA, which suggests that oxidative stress is characteristic of the morbid process *per se*, and that it continues even when the patient has no clinical data on disease progression.

The GSH/GSSG ratio and the antioxidant enzyme activities found in this study are not consistent with the reports of Feijóo, et al.[31], who found that in patients with chronic articular inflammatory disease, these parameters were lower than in control subjects; the authors found differences in oxidative stress between patients with active and inactive disease. Several research groups have found both high and low levels of the antioxidant enzyme activities in RA patients[32,33]. This divergence between reports is frequent and may be explained by the variability and complexity of the regulating mechanism of oxidative stress in humans, which is associated with genetic[2], epigenetic[34], age[35], gender[36], and dietary[37] factors. Thus, it is appropriate to continue this type of study to find a unifying set of markers that could be used in clinical practice[38,39].

The higher GSH/GSSG ratio in RA patients compared with controls, apparently due to higher levels of GSH with no differences in the concentration of GSSG, could be associated to the higher glutathione reductase (GR) activity in RA. Glutathione reductase is a flavoenzyme dependent on NADPH that catalyzes the reduction of GSSH to GSH. This possibility is supported in the study by Feijóo, et al.[31], who found that myeloperoxidase levels are elevated in patients with chronic inflammatory disease, especially those with active disease, and that high myeloperoxidase levels are related to an increase in oxidative damage and the inflammatory response, for myeloperoxidase and GR seem to show a similar activity pattern based on the availability of NADPH. On the other hand, in marine mammals, which have the ability to tolerate the oxidative stress that presumably occurs in the ischemia-reperfusion periods associated to diving apnea, higher GR activity was observed in comparison with non-diving mammals, and this is suggested as one of the adaptive mechanisms of protection against ROS-induced damage[40].

Figure 1. Distribution of the residual values in the lineal model adjusted by the values of DAS28 in individuals with rheumathoid arthritis (RA).



In our study, lower SOD/GPx ratios and higher oxidative damage were found in RA patients. This, according to the study of Sánchez-Rodríguez, et al.[41], suggests a deficiency in the extracellular antioxidant defense system. Similar observations in construct model were reported in patients with chronic articular inflammatory disease in the study by Feijóo, et al.[31].

In the present study, the bivariate correlation model was positive between the GSH/GSSG ratio and the GSH concentration. The multiple linear regression analysis showed a negative association between an inflammatory activity of the disease and the GSSG content. These data support the possibility of a higher GR activity in RA. The only antioxidant enzyme that showed a positive correlation with both lipid peroxidation levels and the antioxidant protection mechanisms was GPx, suggesting that GPx activity is involved in the primary mechanisms against oxidative stress in RA patients. Both GPx and CAT use H_2O_2 as substrate; however, CAT acts in the presence of high concentrations of the substrate while GPx acts at low concentrations, which suggests an inverse correlation between these enzymes and, further, that in RA patients the H_2O_2 concentration may be lower than in other chronic inflammatory diseases, with oxidative damage being mediated possibly by HO•[42].

Multiple linear regression models to assess the effect of the co-variables of age, BMI, Hb concentration, TBARS levels, protein carbonyl content, activity of SOD and GPx, and GSH and GSSG concentrations on the DAS28 index showed that the most adequate construct was the one including BMI, Hb concentration, GPx activity, and GSSG concentration for the subjects with active or inactive RA ($r^2 = 0.481$; $p < 0.01$). The activity of the disease in the model was related to lower BMI and lower Hb content, both described as factors associated to wasting and chronic inflammation characteristic of chronic diseases such as arterial hypertension, diabetes, and RA[43], diseases in which the endothelial damage appears to be the common factor. Vascular endothelium participates in processes such as oxidative stress, inflammation, immune response, thrombosis, vascular remodeling, and apoptosis, depending on the precise equilibrium for cardiovascular health[44]. A unifying theory was recently postulated to suggest that oxidative stress is the most important pathophysiological mechanism that conditions endothelial damage through at least three activation mechanisms, such as the NAD(P)/NAD(P)H oxidase system, xanthine oxidase, and endothelial nitric oxide synthase[45].

Several laboratory analyses are available to assess oxidative stress. These have been classified into five general procedures that quantify: (i) activity of antioxidant enzymes, (ii) concentration of low molecular weight antioxidants, (iii) balance between pro-oxidants and antioxidants, (iv) concentration of oxidants, and (v) concentration of products of oxidative damage[16]. However, according to Sánchez-Rodríguez, et al.[31], these measurements have been used as isolated markers and in a static way, interpreting oxidative stress as an increase in oxidized molecules or the decrease in intra– and/or extracellular antioxidants, without taking into account that oxidative stress integrates the effect of the exposure to oxidants coupled to the antioxidant protective mechanisms *in vivo* in a dynamic manner. This suggests that there may be individuals with high levels of oxidant molecules but an efficient antioxidant response, as well as subjects without elevated oxidant concentrations but with a deficient antioxidant response. Thus, if the pro-oxidant and antioxidant systems are evaluated independently, there may be errors in interpretation of results and limitations to their clinical application. Jones[16] suggests that the increase in pro-oxidants with the consequent damage to macromolecules is not the only form of oxidative stress as sustenance of the disease process, but is a more complex situation which includes a severe disorder in the signaling and homeostatic control of metabolic pathways and organs, affecting specific processes such as cell cycle, apoptosis, immune response, and cell membrane functions.

In this study, SOD and GPx activities were higher in RA patients than in healthy individuals; however, these were not high enough to reduce oxidative damage to lipids and proteins. There were no significant differences between patients with active and inactive RA, suggesting an ongoing oxidative stress process in RA patients. Discrepancy with some of the previous studies may be due to differences in study design and population, which could lead to differences in genetic polymorphisms, epigenetics, diet, sex, or age. Oxidative stress is a dynamic and complex phenomenon, which requires further research and the design of tools for its assessment and clinical application in diagnosis and prognosis of disorders including rheumatoid arthritis.

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REFERENCES

1. Harris ED. Rheumatoid arthritis. Pathophysiology and implications for therapy. N Engl J Med. 1990;322:1277-89.
2. Hitchon CA, El-Gabalawy HS. Oxidation in rheumatoid arthritis. Arthritis Res Ther. 2004;6:265-78.
3. Freire M, Graña J, Galdo F, et al. Guías clínicas: Artritis Reumatoide. Fisterra. 2004;4:1-6.
4. SER. GUIPCAR: Guía de práctica clínica para el manejo de la artritis reumatoide en España: Sociedad Española de Reumatología 2001.
5. Babior BM. Phagocytes and oxidative stress. Am J Med. 2000; 109:33-44.
6. Forman HJ, Fukuto JM, Torres M. Redox signaling: thiol chemestry defines which reactive oxygen and nitrogen species can act as second messengers. Am J Physiol Cell Physiol. 2004;287:C246-56.
7. McCord JM. The evolution of free radicals and oxidative stress. Am J Med. 2000;108:652-9.
8. Henrotin YE, Bruckner P, Pujol JP. The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthritis Cartilage. 2003;11:747-55.
9. Das UN. Free radicals: biology and relevance to disease. J Assoc Physicians India. 1990;38:495-8.
10. Halliwell B. Oxygen radicals, nitric oxide and human inflammatory joint disease. Ann Rheum Dis. 1995;54:505-10.
11. Vasanthi P, Nalini G, Rajasekhar G. Status of oxidative stress in rheumatoid arthritis. Int J Rheum Dis. 2009;12:29-33.
12. Kosower NS, Kosower EM. The glutathione status of cells. Int Rev Cytol. 1978;54:109-60.
13. Pastore A, Piemonte F, Locatelli M, et al. Determination of blood total, reduced, and oxidized glutathione in pediatric subjects. Clin Chem. 2001;47:1467-9.
14. Jones DP. Redox potential of GSH/GSSH couple:assay and biological significance. Methods Enzymol. 2002;348:93-112.
15. Park EM, Ramnath N, Yang GY, et al. High SOD and low GPX activities in RBC predict susceptibility of lung cancer patients to radiation pneumonitis. Free Radic Biol Med. 2007; 42:280-7.
16. Jones DP. Redefining oxidative stress. Antioxid Redox Signal. 2006;8:1865-79.
17. Henrotin YE, Bruckner P, Pujol JP. The role of reactive oxygen species in homeostasis and degradatioin of cartilage. Osteoarthritis Cartilage. 2003;11:747-55.
18. De Leo ME, Tranghese A, Passantino M, et al. Manganese superoxide dismutase, glutathione peroxidase, and total radical trapping antioxidant capacity in active rheumatoid arthritis. J Rheumatol. 2002;29:2245-6.
19. Karakoc M, Altindag O, Keles H, Soran N, Selek S. Serum oxidative-antioxidative status in patients with ankylosing spondilitis. Rheumatol Int. 2008;27:1131-4.
20. Arnet FC. The american rheumatism association 1987 revised criteria for classification of rheumatoid arthritis. Arth Rheum. 1988;31:315-24.
21. Olsson A, Skogh T, Winger G. Comorbidity and lifestyle, reproductive factors, and enviromental exposures associated with rheumatoide arthritis. Ann Rheum Dis. 2001;10:934-9.
22. Smolen JS, Breedveld FC, Ebrel G, et al. Vality and reliability of the twenty-eight-joint count for assesment of rheumatoid arthritis activity. Arthritis Rheum. 1995;38:38-43.
23. NOM-253-SSA1-2012. Para la disposición de sangre humana y sus componentes con fines terapéuticos. Norma Oficial Mexicana. 2012:1-20.
24. Susuki. Measurment of Mn-Sod and Cu, Zn-Sod. In Experimental protocols for reactive oxygen and nitrogen species. Gutteridge J, Taniguchi N (Ed). Oxford, UK. Oxford University Press. 2000; 91-5.
25. Flohé L, Günzler WA. Assays of glutathione peroxidase. Methods Enzymol. 1984;105:114-20.
26. Vázquez-Medina JP, Zenteno-Savín T, Elsner R. Glutathione protection aginst dive-associated ischemia/reperfusion in ringed seal tissues. J Exp Marine Biol Ecology. 2007;345:110-8.
27. Zenteno-Savin T, Clayton-Hernández E, Elsner R. Diving seals: are they a model for coping with oxidative stress? Comp Biochem Physiol C Toxicol Pharmacol. 2002;133:527-36.
28. Levine RL, Williams JA, Satdtman ER, Shaeter E. Carbonyl assays for determination of oxydatively modified proteins. Methods Enzymol. 1994;233:346-57.
29. Gaxiola-Robles R, Bitzer-Quintero OK, Méndez-Rodríguez LC, et al. Lipid peroxidation and the response of the antioxidant defense system in the obese type 2 diabetic compared with non-obese tyupe 2 diabetic. Nutri Hosp. 2013;28:1905-11.
30. Heliovaara M, Knekt P, Aho K, Aran RK, Alfthan G, Aromaa A. Serum antioxidants and risk of rheumatoid arthritis. Ann Rheum Dis. 1994;53:51-3.
31. Feijóo M, Túnez I, Ruiz A, Tasset I, Muñoz L, Collantes E. [Oxidative stress biomarkers as indicator of chronic inflammatory joint diseases stage]. Reumatol Clín. 2010;6:91-4.
32. Ozturk HS, Cimen MY, Cimen OB, Kacmaz M, Durak I. Oxidant/antioxidant status of plasma samples from patients with rheumatoid arthritis. Rheumatol Int. 1999;19:35-7.
33. 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.
34. Sánchez-Pernaute O. [Epigenetic therapies, a step beyond biologics for rheumatoid arthritis]. Reumatol Clin. 2010;6:306-10.
35. Samiec PS, Drews-Botsch C, Flagg EW, et al. Glutathione in human plasma: decline in association with aging, age-related macular degeneration, and diabetes. Free Radic Biol Med. 1998; 24:699-704.
36. Borrás-Blasco C. Importancia del estrés oxidativo en la diferencia de longevidad entre machos y hembras. Tesis doctoral. Facultad de Medicina y Odontología. Universidad de Valencia. España. 2003.
37. Lonn E, Bosch J, Yusuf S, et al. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. JAMA. 2005;293:1338-47.
38. Pérez Gastell PL, Pérez de Alejo JL. Métodos para medir el daño oxidativo. Rev Cubana Med Milit. 2000;29:192-8.
39. Kalpakcioglu B, Senel K. The interrelation of glutathione reductase, catlase, glutathione peroxidase, superoxide dismutase, and glucose-6-phosphate in the pathogenesis of rheumatoid arthritis. Clin Rheumatol. 2008;27:141-5.
40. Vázquez-Medina JP, Zenteno-Savín T, Elsner R. Antioxidant enzymes in ringed seal tissues: potential protection against dive-associated ischemia/reperfusion. Comp Biochem Physiol C Toxicol Pharmacol. 2006;142:198-204.
41. Sánchez-Rodríguez M, Santiago-Osorio E, Vargas L, Mndoza-Núñez V. Propuesta de un constructo para evluar integralmente el estrés oxidativo. Bioquimia. 2004;9:81-90.
42. Cisnero Prego E, Pupo Balboa J, Céspedes Miranda E. Enzimas que participan como barreras fisológicas para eliminar los radicales libres: III. Glutatión peroxidasa. Rev Cubana Invest Biomed. 1997;16:10-5.
43. Pasceri VA. A tale of two diseases: atherosclerosis and rheumatoid arthritis. Circulation. 1999;100:2124-6.
44. Gopaul NK, Manraj MD, Habe A, et al. Oxidative stress could precede endothelial dysfunction and insulin resistance in Indian Mauritians with impaired glucose meatbolism. Diabetologia. 2001;44:706-12.
45. Ceballos-Reyes G, Ramírez-Sánchez I, Calzada-Mendoza C, Olivares-Corichi IM. Disfunción endotelial y estrés oxidativo. Endocrinol Nutr. 2006;14:233-6.