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Assessment of oxidative stress biomarkers in patients before pharmacological treatment for rheumatoid arthritis

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ABSTRACT. Rheumatoid Arthritis (RA) significantly decreases the functional capacity of 1% of the world's population, and it is believed that oxidative stress may be one of its triggering factors. Thus, the present study evaluated the biomarkers of oxidative stress in patients with rheumatoid arthritis before pharmacological treatment and their relationship with the DAS 28 score. This is an observational, cross-sectional study with a quantitative and analytical approach. Patients treated at a rheumatology clinic in the city of Ijuí/RS, with a diagnosis of rheumatoid arthritis, were selected and compared with the control group. Data collection took place between August and September 2020. In the 18 patients who participated in the research, higher values of SOD ($p = 0.045$), NP-SH ($p \leqslant 0.000$) and TBARS ($p = 0.005$), and reduced levels of CAT ($p \leqslant 0.000$) were found, when compared to the control. The enzymatic levels of the patients showed a significant association with body mass index. Furthermore, in this study, the pain complaint did not correlate with oxidative stress markers. This study showed changes in oxidative stress biomarkers in patients with RA before pharmacological treatment, demonstrating that these are indicative of a worse prognosis, which is estimated to be minimized with adequate pharmacological treatment.

Keywords: antioxidants; free radicals; rheumatoid arthritis; pharmacological treatment; catalase; superoxide dismutase.

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by symmetrical lesions and the involvement of the synovial membrane of joints (Mateen, Zafar, Moin, Khan, & Zubair, 2016b). It is estimated that about 0.5 to 1% of the population in developed countries are affected by RA, with women being three times more prone, especially in the 30 to 50 age group (Mota et al., 2012). In Brazil, the prevalence in adults ranges from 1 to 2%, equivalent to 1.3 million people (Marques-Neto et al., 1993; Álvarez-Hernández et al., 2012).

The RA has unknown causes, but advances have been observed in the knowledge of its pathophysiological mechanisms (McInnes & O'Dell, 2010). This disease causes several physiological changes in the patient and can trigger comorbidities, which are supposed to have oxidative stress as one of the triggering factors (Fonseca, Nunes-Souza, Goulart, & Rabelo, 2019).

According to a study carried out by Ali, Habeeb, El-Azizi, Khattab, Abo-Shady and Elkabarity (2014) in Egypt, significant amounts of reactive oxygen species (ROS) were found in the synovial fluid of inflamed joints of patients with RA. Other research shows that people with this disease also show an increase in protein oxidation, lipid peroxidation and deoxyribonucleic acid (DNA) damage, as well as a decrease in plasma levels of antioxidants (Mateen, Moin, Khan, Zafar, & Fatima, 2016a).

The main strategy to control RA symptoms is the use of pharmacological treatment, including: non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, synthetic and biological disease-modifying drugs (DMARDs), as well as immunosuppressive drugs (Mota et al., 2012). All these agents have been shown to be effective in reducing oxidative stress (Hirao et al., 2012), and the initial period of the disease is critical, as earlier intervention tends to be more effective in minimizing stress-related damage and improving the clinical outcome (McInnes & O'Dell, 2010).

To confirm the above, a study in Spain (Feijóo et al., 2009) analyzed eighteen untreated RA patients and showed that this group had an increase in oxidative damage to DNA and lipid peroxidation, when compared to control group. After treatment with antirheumatic drugs, these levels reduced considerably.

In addition to changing levels of antioxidants and reactive species in patients with RA, these parameters also seem to be related to the disease activity itself (Seven, Güzel, Aslan, & Hamuryudan, 2008), although some results are still controversial. Studies have shown a positive correlation between myeloperoxidase levels and the DAS 28 score (Stamp et al., 2012), and in patients with active disease, nitric oxide serum levels are higher (Ali et al., 2014). In comparison, when stratified, patients with DAS greater than 2.7 showed higher levels of TBARS, lipid hydroperoxide and conjugated diene, while lower levels of glutathione, GSH Px, SOD and catalase were found in this group (Seven et al., 2008). In contrast, a 2021 study revealed positive, but not significant, correlations between DAS 28 and TBARS concentration (Šteňová, Bakošová, Lauková, Celec, & Vlková, 2021).

However, despite the recognized association between oxidative stress and RA, studies with this approach remain scarce, particularly with data collected from Brazilian patients. Therefore, this study aims to update and expand upon existing information, as well as potentially contribute to the early diagnosis and evaluation of disease activity, aiming to help in choosing its treatment, considering that this morbidity significantly reduces the functional capacity of affected individuals and increases morbidity and mortality rates, resulting in substantial costs for the healthcare system (Goeldner, Skare, Reason, & Utiyama, 2011).

In view of the above, the present study intends to evaluate the biomarkers of oxidative stress in patients with RA at the beginning of treatment, as well as to analyze their relationship with clinical variables, medication use and DAS 28 score.

Material and methods

This is an observational, cross-sectional study, with a quantitative and analytical approach, to assess the oxidative stress profile of patients at a rheumatology clinic in the city of Ijuí, Rio Grande doSul state.

The inclusion criteria for the sample consisted of patients who were newly diagnosed with RA, had not received continuous treatment previously prescribed by a medical professional for this condition, had a reactive C-reactive protein (CRP) test result, were over 18 years old, and provided their consent to participate in the study by signing the Informed Consent Form.The sample exclusion criteria were patients who had other inflammatory diseases or who did not accept to participate in the research.

Sampling was intentional and 18 patients were selected in the first consultation with the rheumatologist and with a clinical diagnosis of RA, later confirmed with CRP test. The results were compared with a control group, composed of 18 individuals, matched for gender and age, with no diagnosed comorbidities, and no use of medication or any inflammatory problem.

Data collection took place between August and September 2020, using a questionnaire with objective and descriptive questions to assess sociodemographic and anthropometric information, as well as information related to the patient's behavior and health status. Two tubes of 4 ml venous blood with EDTA were collected for analysis of enzymes and oxidative stress markers by a pharmacist, taking into account all relevant biosafety standards. The application of the instruments and collection of samples from the patients with RA took place at the aforementioned clinic, and at home for the control patients, with the same collection procedure performed for both groups. Data analysis was carried out in October 2020.

For the analyzes of oxidative stress biomarkers, vacuum tubes of ethylene-diamine-tetra-acetic acid (EDTA) were used to obtain erythrocytes (RBC), in which whole blood samples were centrifuged for 15 minutes at 2500 rpm, washed twice in 0.9% sodium chloride and recovered by centrifugation.

The catalase activity (CAT) in red blood cells was measured using the Aebi method. Erythrocytes were added to a cuvette with 50 mM phosphate buffer (pH 7.0) and the reaction was initiated by adding freshly prepared 0.5 mM hydrogen peroxide (pH 7.0). The decomposition rate of hydrogen peroxide was then measured by a spectrophotometer at 240 nm (Aebi, 1984).

Superoxide dismutase (SOD) activity was analyzed by the method described by McCord and Fridovich, which is based on the ability of SOD to inhibit the auto-oxidation of adrenaline to adrenochrome. The test was performed with a diluted solution of erythrocytes, using three volumes, being read in a spectrophotometer at 480 nm (McCord & Fridovich, 1969).

The non-protein thiol groups (NP-SH) of red blood cells, which allow to indirectly check glutathione (GSH) levels, were determined using RBC hemolyzed with 10% Triton. To this mixture, 20% trichloroacetic acid (TCA) was added, which was then centrifuged at 4000 rpm for 10 minutes. The supernatant was used as a sample for performing the standard curve, using different concentrations of 1 mM GSH. Then, 5',5'-dithio-

bis-(2-nitrobenzoic acid) (DTNB) was added to the mixture, which was immediately read in a spectrophotometer at 412 nm (Boyne & Ellman, 1972).

The RBC were used to analyze the species reactive to thiobarbituric acid (TBARS). This preparation was carried out with the addition of 10mM butylhydroxytoluene (BHT) and 20% TCA, and then homogeinized in vortex and centrifuged for 5 minutes at 4000 rpm. With the supernatant, a standard curve was performed using different concentrations and volume of distilled water, malondialdehyde (MDA) 0.03 mM, phosphoric acid (H3PO4) 10% and TBA 0.6%. The tubes were placed in a water bath at 95ºC for 60 minutes and immediately read in a spectrophotometer at 532 nm (Moore, Brummitt, & Mankad, 1989).

For conventional biochemical analyses, the following reference values were used: creatinine from 0.60 to 1.30 mg dL^{-1}; gamma-glutamyltranspeptidase (Gamma-GT) < 55 U L^{-1}; alkaline phosphatase from 25 to 100 U L^{-1}; glutamic oxaloacetic transaminase (TGO/AST) < 40 U L^{-1}; glutamic pyruvic transaminase (TGP/ALT) < 41 U L^{-1}; total cholesterol < 190 mg dL^{-1}; high-density lipoprotein (HDL) > 40 mg dL^{-1}; low-density lipoprotein (LDL) < 130 mg dL^{-1}; triglycerides < 150 mg dL^{-1} (Abadie, 2024). These analyzes were carried out in an outsourced laboratory during the same period of data collection.

The drugs used by the study population were classified according to the Anatomical Therapeutic Chemical Classification (ATCC) (World Health Organization [WHO], 2023), first and second level. The calculation and classification of the Body Mass Index (BMI) was carried out in accordance with the recommendations of the Ministry of Health: a) underweight (less than 18.5); b) normal weight (between 18.5 and 24.9); c) overweight (between 25 and 29.9); d) obesity (equal to or above 30).

To assess disease activity, the Disease Activity Score (DAS 28) was used. This score evaluates the clinical status of patients and comprises four variables: painful and swollen joints comprising 28 joints, C-reactive protein (CRP) and pain intensity through the Visual Analog Pain Scale (VAS) (Gestel, Haagsma, & Riel, 1998).

The curves were created using the Microsoft Excel program and the other data were analyzed using the Statistical Package for Social Sciences (SPSS) software (version 18.0). Descriptive analysis was presented as mean and standard deviation, relative and absolute frequency, and associations between groups were analyzed using Student's t test for paired and independent samples. In order to verify the association between 3 or more groups, the one-way ANOVA test was used, followed by Tukey's post hoc test.

The study was approved by the Research Ethics Committee (CEP) of the Regional University of the Northwest of the State of Rio Grande do Sul (UNIJUÍ), with opinion number 4.019.693/2020.

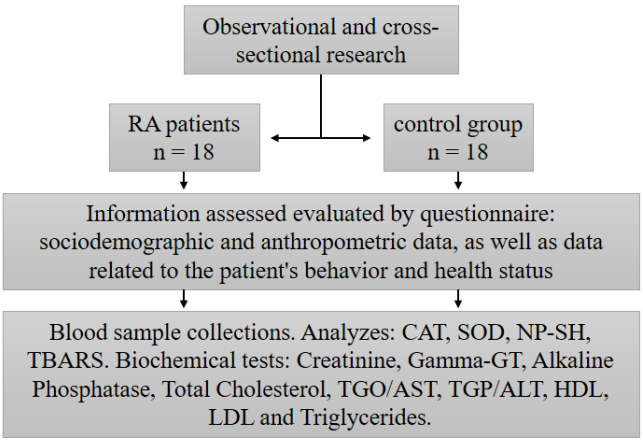

Figure 1. Research and data collection flowchart.Legend: (AR) Rheumatoid Arthritis; (CAT) Catalase; (SOD) Superoxide Dismutase; (NP-SH) Non-Protein Thiols; (TBARS) Thiobarbituric Acid Reactive Species; (HDL) High Density Lipoprotein; (LDL) Low Density Lipoprotein; (TGO/AST) Glutamic oxaloacetic transaminase; (TGP/ALT) Glutamic Pyruvic Transaminase; (Gamma-GT) Gamma-glutamyltranspeptidase.

Results and discussion

A total of 36 individuals participated in the study, of which 18 were from the group of patients with RA and 18 from the control group. In the RA group, 17 (94.4%) were female, with a mean age of 56.05 ± 10.3 years; 11 (61.1%) declared themselves white and 7 (38.9%) brown; 14 (77.8%) had low education level; 12 (66.7%) were married or with a partner and 16 (88.9%) lived with a partner and/or children. Regarding the control group, it is also composed of 17 (94.4%) women, with a mean age of 56.61 ± 11.0

years; 14 (77.8%) recognized themselves as white, 3 (16.7%) brown and 1 (5.6%) black. Regarding sociodemographic characteristics, there was no significant difference between the groups regarding age (p = 0.246), sex (p = 0.817) and skin color (p = 0.667).

Among the patients included in this study, 11 (61.1%) had associated comorbidities, of which the most frequent was systemic arterial hypertension (SAH) (44.4%), followed by psychological/psychiatric disorders (27.8%) and hypercholesterolemia (16.7%). Regarding medication, 12 (66.7%) patients reported the occasional use of analgesics and anti-inflammatories for the symptoms of RA. These same patients were also in use of continuous treatments for associated comorbidities, with prevalence of psycholeptics (44.4%), followed by other categories (Table 1).

Table 1. Clinical profile of patients starting treatment for rheumatoid arthritis followed by a rheumatology clinic in the city of Ijuí, RS, 2020. (N = 18).

Variables	Categories		n (%)
Associated Comorbidities	Yes		11 (61.1)
	No		7 (38.9)
Types of Comorbidities	HAS		8 (44.4)
	Psychological/Psychiatric		5 (27.8)
	Hypercholesterolemia		3 (16.7)
	DM		2 (11.1)
	Hypothyroidism		1 (5.6)
	Cardiovascular diseases		1 (5.6)
	Drop		1 (5.6)
Use of occasional and continuous medication	Yes		12 (66.7)
	No		6 (33.3)
Class of drugs according to the Anatomical Therapeutic Chemical Classification (ATCC) first and second level	1st Level	2nd Level	
	A - Digestive system and metabolism	A02 Antacids	5 (27.7)
		A10 Antidiabetics	2 (11.1)
	C - Cardiovascular System	C02 Antihypertensives	6 (29.0)
	M - Musculoskeletal System	M01 Anti-inflammatories	7 (33.8)
		M04 Antigut	1 (5.6)
	N – Nervous System	N02 Analgesics	7 (33.8)
		N03 Antiepileptic	1 (5.6)
		N05 Psycholeptics	8 (44.4)

Legend: (SAH) Hypertension; (DM) Diabetes Mellitus; (RA) Rheumatoid Arthritis.

Regarding the anthropometric characterization, no statistical significance was observed between the groups, as shown in Table 2. However, the BMI value of 14 (77.7%) RA patients and 13 (72.2%) controls showed signs of overweight.

Table 2. Anthropometric characterization of controls and patients starting treatment for rheumatoid arthritis followed by a rheumatology clinic in the city of Ijuí, RS, 2020. (Control Group N = 18, Patients with RA N = 18).

Variables	CG	PAR	p values*
Weight (kg)	76.66 ± 14.47	69.17 ± 20.66	0.841
Height (m)	1.69 ± 0.08	1.51 ± 0.38	0.534
BMI (kg m^{-2})	26.26 ± 3.45	26.92 ± 7.55	0.638

*p < 0.05 in relation to the CG by Student's t test analysis. Legend: (BMI) Body Mass Index; (CG) Control Group; (PAR) Patients with RA.

The biochemical parameters of the control group and RA patients were compared using their respective me ans, and the results showed no statistical significance. However, it is worth mentioning that the average HDL value of the RA patients is lower than the recommended by the Brazilian Society of Clinical Analyzes (Abadie, 2024), (Table 3).

Table 3. Analysis of biochemical parameters of controls and patients starting treatment for rheumatoid arthritis followed by a rheumatology clinic in the city of Ijuí, RS, 2020. (Control Group N = 18, Patients with RA N = 18).

Parameters	CG	PAR	p values*	Reference values **
Triglycerides (mg dL^{-1})	113.61 ± 75.96	119.05 ± 254.92	0.398	< 150 mg dL^{-1}
Total Cholesterol (mg dL^{-1})	187.28 ± 71.63	173.25 ± 61.75	0.274	< 190 mg dL^{-1}
HDL cholesterol (mg dL^{-1})	54.05 ± 11.61	20.11 ± 26.89	0.056	> 40 mg dL^{-1}
LDL cholesterol (mg dL^{-1})	127.00 ± 47.27	121.57 ± 31.43	0.529	< 130 mg dL^{-1}
Creatinine (mg dL^{-1})	1.06 ± 0.24	0.83 ± 0.22	0.235	0.60 a 1.30 mg dL^{-1}

Alkaline Phosphatase (U L^{-1})	70.83 ± 32.70	87.00 ± 29.00	0.670	25 a 100 U L^{-1}
TGO/AST (U L^{-1})	22.50 ± 7.31	20.00 ± 10.34	0.877	< 40 U L^{-1}
TGP/ALS (U L^{-1})	20.78 ± 10.34	26.22 ± 34.69	0.367	< 41 U L^{-1}
Gamma-GT (U L^{-1})	26.22 ± 8.04	19.33 ± 40.16	0.423	< 55 U L^{-1}

*p < 0.05 compared to control by Student's t-test analysis for paired samples.**Reference values (Sociedade Brasileira de Análises Clínicas, 2011).Legend: (CG) Control Group; (PAR) Patients with RA; (HDL) High Density Lipoprotein; (LDL) Low Density Lipoprotein; (TGO/AST) Glutamic oxaloacetic transaminase; (TGP/ALT) Glutamic Pyruvic Transaminase; (Gamma-GT) Gamma-glutamyltranspeptidase.

As for markers of oxidative stress, the results indicate that SOD (p = 0.045), NP-SH (p ⩽ 0.001) and TBARS (p = 0.005) levels are increased in RA patients, and CAT (p ⩽ 0.001) is reduced in relation to the control group (Table 4).

Table 4. Levels of oxidative stress biomarkers in controls compared to patients starting treatment for rheumatoid arthritis followed by a rheumatological clinic in the city of Ijuí, RS, 2020. (Control Group N = 18, Patients with RA N = 18).

Variables	CG	PAR	p values*
CAT (mmol $H_2O_2^{-1}$ mL^{-1} eri)	88.43 ± 27.52	71.32 ± 27.08	0.001*
SOD (μSOD mL^{-1} Hb)	179.75 ± 130.80	249.53 ± 166.37	0.045*
NP – SH (nmol NP-SH mL^{-1} eri)	1456.96 ± 571.99	2288.78 ± 864.59	0.001*
TBARS (nmol MDA mL^{-1} eri)	2.97 ± 1.58	5.13 ± 1.27	0.005*

*p < 0.05 compared to control by Student's t-test analysis.; Legend: (CG) Control Group; (PAR) Patients with RA; (CAT) Catalase; (SOD) Superoxide Dismutase; (NP-SH) Non-Protein Thiols; (TBARS) Thiobarbituric Acid Reactive Species.

Table 5 shows that CAT (p = 0.033) and TBARS (p = 0.047) levels are significantly lower in patients with normal BMI, when compared to those with obesity. The variables skin color, associated comorbidities, and use of occasional and continuous medication did not show influence on biomarkers of oxidative stress, both evaluated in the control group and in the rheumatoid arthritis group.

The DAS 28 classification, which assesses disease activity, had an average of 4.31 ± 0.77, with a maximum of 5.8 and a minimum of 2.90. In Spearman's correlation analysis, the DAS 28 results correlated positively with TBARS (r = 0.085, p = 0.74), and negatively with CAT (r = -0.29, p = 0.25), NP- SH (r = -0.10, p = 0.70) and SOD (r = -0.22, p = 0.54), however, none of these showed statistical correlation.

Differences between whites and browns were evaluated, and these did not show statistical significance when associated, according to BMI classification (p = 0.351). In the brown group, 57.5% had comorbidities and 71.4% were overweight/obese.

The study predominantly consisted of women, which is consistent with the literature showing a prevalence ratio of 5.2 women to each man for RA in Latin America (Barragán-Martínez et al., 2012). Evidence supports that women are less susceptible to increased oxidative stress due to the antioxidant properties of female hormones, such as estrogen. Nonetheless, factors as menopause, increasing age and metabolic decline contribute to the imbalance of antioxidant compounds (Kander, Cui, & Liu, 2017), which is in line with the average age of the patients in the present study. There was no association between age and biomarkers of oxidative stress, as in the cross-sectional study by García-González, Gaxiola-Robles and Zenteno-Savín (2015) in Mexico with 29 newly diagnosed patients, which is relevant since age may influence glycolytic and oxidative metabolism (Tromm et al., 2018).

In the present study, part of the sample self-identified as brown presented higher NP-SH levels, although without statistical difference. According to the literature, approximately 90% of pardos/blacks carry at least one polymorphic allele associated with RA, which makes them four times more likely to develop the disease (Govind et al., 2019). However, in this study, this group does not comprise the majority, which can be explained by data from the Brazilian Institute of Geography and Statistics (Instituto Brasileiro de Geografia e Estatística [IBGE], 2010) indicating that in the southern region of Brazil, whites represent 78.5% of the population, and browns, blacks and other ethnic groups represents only 21.5%. About the association between oxidative stress and skin color, few published studies were found, but it is believed that the black/brown population has a homeostatic imbalance that leads to increased platelet aggregation, providing a prothrombotic state and decreased antioxidant capacity as a result of this event (Myburgh, Huisman, & Mels, 2019), which may justify the results of this work, in which 57.5% of this group presented comorbidities and 71.4% had overweight/obesity. Increased levels of NP-SH, on the other hand, may be related to a physiological quest to restore redox balance (Lammertyn, Mels, Pieters, Schutte & Schutte, 2015).

Table 5. Association of variables with biomarkers of oxidative stress in controls and patients starting treatment for rheumatoid arthritis followed by a rheumatology clinic in the city of Ijuí, RS, 2020. (Control Group N = 18, Patients with RA N = 18).

	Variables			Mean ± Standard Deviation		p* values	
	BMI classification #					Intragroup	
		GC	*PIR*	*GC*	*PAIR*	*GC*	*PAR*
CAT	Normal	5	3	73.45 ± 26.32	**48.07 ± 8.72**		
	Overweight	11	8	100.02 ± 24.37	61.50 ± 13.70	0.070	0.033*
	Obesity	2	6	64.68 ± 30.80	**89.60 ± 32.68**		
SOD	Normal	5	3	163.35 ± 115.06	121.67 ± 106.91		
	Overweight	11	8	206.42 ± 148.78	225.84 ± 86.15	0.656	0.282
	Obesity	2	6	103.68 ± 59.44	301.91 ± 227.47		
NP-SH	Normal	5	3	1382.53 ± 364.28	1825.09 ± 558.63		
	Overweight	11	8	1545.56 ± 686.04	2338.07 ± 482.78	0.665	0.685
	Obesity	2	6	1156.45 ± 403.05	2321.43 ± 1352.67		
TBARS	Normal	5	3	2.28 ±0.65	**3.64 ± 1.83**		
	Overweight	11	8	3.5 ±1.81	5.08 ± 0.77	0.208	0.047*
	Obesity	two	6	1.92 ±0.43	**5.76 ± 1.06**		
	Age &						
CAT	< 60	10	12	82.13 ± 28.70	74.10 ± 30.25	0.482	0.219
	> 60	8	6	96.30 ± 25.54	65.75 ± 20.61		
SOD	< 60	10	12	155.87 ± 119.64	250.47 ± 187.84	0.531	0.437
	> 60	8	6	209.59 ± 145.98	247.63 ± 128.33		
NP-SH	<60	10	12	1254.00 ± 580.27	2265.76 ± 998.25	0.718	0.403
	>60	8	6	1710.66 ± 478.78	2333.91 ± 587.79		
TBARS	<60	10	12	2.65 ± 1.64	5.15 ± 1.43	0.798	0.564
	>60	8	6	3.38 ± 1.50	5.11 ± 0.94		
	Skin color #						
CAT	White	14	11	88.96 ± 25.86	78.94 ± 32.39	0.432	0.139
	Brown	3	7	76.00 ± 37.50	59.34 ± 7.41		
	Black	1	0	118.34	-		
SOD	White	14	11	160.70 ± 110.19	268.70 ± 211.03	0.108	0.556
	Brown	3	7	180.72 ± 170.83	219.38 ± 49.59		
	Black	1	0	443.46	-		
NP-SH	White	14	11	1421.86 ± 588.99	2270.82 ± 1089.24	0.499	0.917
	Brown	3	7	1393.95 ± 529.01	2316.22 ± 372.95		
	Black	1	0	2137.51	-		
TBARS	White	14	11	2.91 ± 1.63	5.18 ± 1.59	0.460	0.876
	Brown	3	7	2.62 ± 1.38	5.07 ± 0.55		
	Black	1	0	4.19	-		

*p < 0.05 for statistical test, within the control group and within the arthritis group. & Student's t test for independent samples. # one-way anova test, followed by Tukey's post hoc test (values with statistical difference high lighted in bold). Legend: (CG) Control Group; (PAR) Patients with RA; (BMI) Body Mass Index; (RA) Rheumatoid Arthritis; (CAT) Catalase mmol $H_2O_2^{-1}$ mL^{-1} eri; (SOD) Superoxide Dismutase μ SOD mL^{-1} Hb; (NP-SH) Non-Protein Thiols nmol NP-SH mL^{-1} eri; (TBARS) Thiobarbituric Acid Reactive Species nmol MDA mL^{-1} eri.

Most women in the study reported comorbidities, with the most frequent being systemic arterial hypertension (SAH), psychological/psychiatric disorders, and hypercholesterolemia. Studies show that patients with RA have a higher prevalence of hypertension and dyslipidemia than the general population (Peters & Nurmohamed, 2013; Castro, Lanna, Rocha, Ribeiro, & Telles, 2018). The presence of these diseases in patients with RA accelerates the process of atherogenesis and recruits cytokines that contribute to the onset of oxidative stress (Brenol, Monticielo, Xavier, & Brenol, 2007). Psychological and psychiatric disorders, such as depression, are commonly associated with diseases that affect quality of life, such as RA (Nerurkar, Siebert, McInnes, & Cavanagh, 2019). Nevertheless, the mechanisms that link depression, inflammation and oxidative stress are not fully understood, although it has been reported that the brain is vulnerable to oxidative damage due to the high use of oxygen and production of neurotoxic metabolites, which contribute to the generation of free radicals and production of pro-inflammatory cytokines (Lindqvist et al., 2017). However, this study did not find any statistically significant association between comorbidities and oxidative stress. There is also limited research available in the literature regarding this relationship. Nakajima et al. (2014), in an observational study with 150 patients with RA and Diabetes Mellitus (DM), noticed that those with associated DM had lower levels of ROS, which suggests that patients with RA and other concomitant health problems may have worse levels of oxidative stress.

In this study, most patients reported the occasional use of analgesics and anti-inflammatory drugs, as well as continuous use medication for other associated comorbidities, with the most common class being psycholeptics. NSAIDs and simple analgesics are the main classes of drugs recommended to control the symptoms associated with RA, with the ability to relieve pain and discomfort (Comissão Nacional de Incorporação de Tecnologias no Sistema Único de Saúde [CONITEC], 2019). The use of psycholeptics, such as antidepressants and anxiolytics, aims to mitigate the indirect negative effects of depression, both in terms of functional progression and treatment response (Nerurkar et al., 2019). In this study, the use of these agents was not found to be statistically significant when associated with biomarkers of oxidative stress, indicating that they did not influence the results of the analysis. Similar results were obtained in the research conducted by Thiele et al. (2015), in which they observed the lipid peroxidation levels of 80 patients with RA in comparison to a healthy group control, and the use of medications also did not interfere with their result.

When comparing anthropometric characteristics, there was no significant difference within the control group. However, CAT and TBARS levels are significantly lower in patients with normal BMI, compared to those with BMI values above the recommended. Findings reveal a positive correlation between BMI/body fat and inflammation. Research has shown that for every 5 kg/m2 increase in BMI, the risk of developing RA increases by 8%, with a higher risk in women than in men (Feng et al., 2019). Likewise, studies also indicate that overweight/obesity is associated with inflammation and oxidative stress, since increased body fat activates the inflammatory cascade, which secretes adipokines responsible for inducing the production of free radicals, which cause DNA damage, lipid and protein peroxidation (Mishra et al., 2012; Fonseca et al., 2019).

There was no statistically significant difference in the association betweem the average values of the biochemical exams of the control group and the RA patients, although low HDL average values were verified in these patients. The literature suggests that RA patients have impaired HDL function due to chronic inflammation and disease activity, leading to a decrease in total antioxidant capacity and lipid peroxidation. In the same manner, Rosso et al. (2014), also found reduced HDL values when investigating 12 women with RA in Argentina, suggesting that this decrease may be related to the function of this molecule to protect LDL from oxidative stress. However, in this study, no significant changes were observed in LDL or the other analyzed variables when comparing both groups. Therefore, it can be inferred that the differences in oxidative stress are related to the inflammatory damage caused by RA and not the biochemical changes in these patients. Alternatively, it is possible that the observed oxidative stress is not a result of the aforementioned changes.

When analyzing the participants' oxidative stress biomarkers, it is observed that the average SOD enzymatic activity, NP-SH and TBARS levels are significantly increased in the RA group, in comparison to the control group. According to the literature, the increase in SOD in patients with RA can be explained by the fact that this enzyme is the first one recruited to defend against cellular stress in a pro-inflammatory environment, with the aim of obtaining more stable and less harmful products. The increase in TBARS may be related to the high amount of ROS present in the joint, which, when not eliminated, lead to lipid peroxidation, in which fatty acids are oxidized, causing damage to the cell membrane (Veselinovic et al., 2014). García-González et al. (2015) found significant differences in SOD, glutathione reductase (GSH-Rd), GSH-Px and TBARS for RA patients, when compared to control groups. This may justify that, regardless of the antioxidant levels being higher in these patients, these were insufficient to contain oxidative damage.

Contrary to our findings, Staroń, Mąkosa and Koter-Michalak (2012) found decreased concentrations of SH groups by 30% in 25 patients with newly diagnosed RA compared to controls in their study in Poland. They attributed this to the fact that this group of thiols may be involved in the formation of disulfide bridges aggregated in the walls of membranes, increasing the permeability of ions. However, Pedersen-Lane, Zurier and Lawrence (2007) observed increased levels of SH groups in 117 patients with RA compared to healthy controls. They justified these results by explaining that the cells are constantly striving to minimize the presence of oxidizing compounds in the pro-inflammatory environment of the body, thereby protecting against more severe forms of stress.

On the other hand, the CAT values of the RA patients in this study were significantly lower when compared to the controls. During the elimination process of ROS in inflammation, this enzyme is degraded in order to convert hydrogen peroxide (H_2O_2) into water and oxygen, to protect cells from the harmful effects of accumulated H_2O_2, which may explain its decrease (Mateen et al., 2016a). Other studies have reported that the decrease in CAT levels can be attributed to the H_2O_2 inactivation process, but also suggest that it may be linked to an increase in tissue lipid peroxidation (MDA). Therefore, this enzyme may play a critical role in protecting against the resulting damage (Barbary, Khalek, Elsalawy, & Hazaa, 2011; Shah, Wanchu, &

Bhatnagar, 2011). The damage caused by oxidative stress can harm different cellular structures, such as DNA, carbohydrates, proteins and lipids, in addition to causing changes in growth, differentiation, chemotaxis and cell death (Fonseca et al., 2019). However, the use of medications in this population can enhance antioxidant defenses, thus regulating these physiological changes (Feijóo et al., 2009; McInnes & O'Dell, 2010). The RA is characterized by an unregulated inflammatory process in the synovial fluid, which causes pain and disability (Gibofsky, 2014). Several studies have suggested a relationship between oxidative damage and the maintenance of this inflammatory process (Filippin, Vercelino, Marroni, & Xavier, 2008). Disease activity scores, such as the DAS 28, use the number of painful joints and swollen joints, among other parameters, in their calculation (CONITEC, 2019). Therefore, we used the DAS 28 score for our analysis in this study. However, although the literature has demonstrated a direct relationship between OS markers and disease activity (DAS 28) (Seven et al., 2008; Stamp et al., 2012; Ali et al., 2014; Šteňová et al., 2021), our results did not show the same behavior, possibly due to the small sample size.

This study found changes in oxidative stress biomarkers in patients with RA prior to receiving treatment, as these biomarkers are known to be important indicators of a poor prognosis, which can be improved with appropriate pharmacological treatment. Furthermore, this study explored the potential of oxidative stress tests as co-auxiliary tools for the diagnosis of RA, since previous studies have suggested a positive correlation between disease severity and oxidative stress levels.

However, this study has some limitations that need to be taken into account: 1) the use of occasional and continuous medication that was not controlled; 2) the age differences between patients in the group; 3) there may be other risk factors which influence oxidative stress, and that were not controlled in this study; 4) the lack of comparison data with post-treatment; 5) small sample size, which can limit statistical inferences.

The findings of this research contribute to the literature and to the clinical and therapeutic conducts concerning this population. This study aims to provide new information that can promote evidence-based decison-making. Thus, further studies are recommended to assess the levels of oxidative stress in patients with RA, in order to elucidate the consequences of stress on the progression of disease activity, the potential harm it can cause, and provide insights for selecting the most effective treatment strategies.

Conclusion

AR patients had altered levels of oxidative stress markers. The disease caused an increase in lipid peroxidation, SOD and NP-SH levels and a decrease in CAT. in addition, no significant correlation was observed between the DAS 28 score and the oxidative stress biomarkers tested in the study.

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