
Is fibromyalgia-related oxidative stress implicated in the decline of physical and mental health status?

M. La Rubia¹, A. Rus¹, F. Molina², M.L. del Moral¹

¹Department of Experimental Biology, and

²Department of Health Science,
University of Jaén, Jaén, Spain;

Mercedes La Rubia

Alma Rus, PhD,

Francisco Molina, PhD,

Maria Luisa del Moral, MD

Please address correspondence to:

Alma Rus,

Department of Experimental Biology

(Building B-3), University of Jaén,

Campus Las Lagunillas s/n,

23071 Jaén, Spain.

E-mail: mrus@ujaen.es

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ABSTRACT

Objective. *Fibromyalgia (FM) is a form of non-articular rheumatism characterised by chronic widespread musculoskeletal aching. Although some works have investigated the possible role of oxidative stress in the pathophysiology of FM, none has analysed a significant number of oxidative markers in the same patients. Consequently, we have performed an exhaustive study of the oxidative/antioxidative status in FM patients and healthy controls, as well as the relationship with FM clinical parameters.*

Methods. *In 45 female patients and 25 age-matched controls, we investigated the oxidative (lipid and protein peroxidation, and oxidative DNA damage) and antioxidative status (total antioxidant capacity (TAC), and antioxidant enzyme activities and compounds). Functional capacity and musculoskeletal pain were assessed by Fibromyalgia Impact Questionnaire (FIQ) and Visual Analogue Scale (VAS), respectively. The physical (PCS-12) and mental (MCS-12) health status was evaluated by SF-12.*

Results. *A significant increase in oxidative DNA damage and protein carbonyl content was found in FM patients vs. controls, as well as in antioxidant compounds such as copper and ceruloplasmin. Patients had diminished levels of TAC and zinc. Enzyme activities of superoxide dismutase, glutathione peroxidase, and catalase were lower in FM patients. Significant correlations were observed in patients between oxidative DNA damage and MCS-12, and zinc and PCS-12.*

Conclusion. *These findings reveal an imbalance between oxidants and antioxidants in FM patients. The lower antioxidant enzyme activities may lead to oxidative stress through the oxidation of DNA and proteins, which may affect the health status of FM patients.*

Introduction

Fibromyalgia (FM) is a condition of chronic musculoskeletal pain and fatigue with significantly impaired function and quality of life. FM affects an estimated 3.4–4.9% of adult women and 0.5–1.6% of adult men in the general population (1). Particularly, the estimated prevalence of FM in the adult Spanish population is 2.4% for both sexes, 4.2% for women and 0.2% for men (2). The impact of FM on society includes increased utilisation of health care resources, loss of work productivity, disability, and insurance costs.

Since the aetiology and pathogenesis of FM are still unknown, treatments can be only aimed at improving the symptoms without targeting the underlying pathology, making such treatments rather ineffective. In recent years, genetic, immunogenic, endocrine, neurological, and psychological studies have been performed attempting to clarify the pathogenesis of FM (3,4). Although the cause/s of FM remains to be determined, oxidative stress has been lately suggested to play an important role in FM pathophysiology (5–13). However, some results of these studies have appeared to be ambiguous.

Oxidative stress is the result of imbalance between reactive oxygen species (ROS) and antioxidants in the cells. ROS are highly reactive chemical species with an unpaired electron. ROS have been suggested to play important roles in rheumatologic conditions like chronic fatigue syndrome (CFS), rheumatoid arthritis, and ankylosing spondylitis (14–16). Excessive production of ROS can damage most cellular components, including lipids, proteins, and nucleic acids (17). Lipid peroxidation reflects a chain reaction between polyunsaturated fatty acids and ROS producing lipid peroxides and hydrocarbon polymers that are both highly toxic to the cell (18). Protein oxidation

is an early indicator of tissue damage and the formation of protein carbonyl derivatives is associated with pathological conditions both in humans and in animal models (19). Finally, oxidative damage to DNA has also been proposed to play an important role in a number of pathological disorders (20).

However, these adverse effects can be counteracted by the enzymatic and non-enzymatic antioxidants, which neutralise excess ROS, or oppose their actions. Major enzymatic antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (21). SOD rapidly and specifically reduces superoxide radicals to hydrogen peroxide (H_2O_2), while H_2O_2 is decomposed to water by both GPx and catalase. On the other hand, non-enzymatic antioxidants include zinc, copper, ceruloplasmin, iron, transferrin, ferritin, bilirubin, uric acid, and albumin, and may be influenced by dietary intake of antioxidants, vitamins, and minerals. Zinc plays an essential role for many antioxidant enzymes as co-factor (22), while copper, essential in all plants and animals, it is transferred by albumin and carried to the liver where it is formed into ceruloplasmin. Ceruloplasmin is a glycoprotein that transports 95% of copper in blood and has a major role in the metabolism of copper to which it binds reversibly. Regarding iron and iron stores, this mineral is indispensable for a number of enzymes involved in neurotransmitter synthesis. Transferrin is a major antioxidant protein, whose main function is the transport of iron to proliferating cells. It also acts as an antioxidant by reducing the concentration of free ferrous ion. Finally, ferritin, an iron-binding protein, regulates iron storage and homeostasis.

Although the mechanisms by which oxidative stress can alter nociception are still unclear, several works have suggested a relationship between oxidative stress and pain perception (23-25), the main symptom of patients with FM. In this sense, a critical role for superoxide has been shown in the development of pain by peripheral and central nervous system sensitisation (26). ROS have also been involved in NMDA-receptor activation, an essential step in central

sensitisation, contributing to neuropathic pain (27). On the other hand, oxidative damage has been reported to interfere with the muscles by reducing local nociceptors, causing a diminution in the pain threshold (14).

A number of works have examined the role of oxidative stress in the pathophysiology of FM; however, none has investigated a significant number of oxidative markers in the same patients. In this context, we propose to perform an exhaustive study of the oxidative and antioxidative status in patients with FM and healthy controls with the aim of evaluating the potential role of oxidative stress in the pathophysiology of FM, as well as identifying reliable biomarkers of disease. Finally, we aim to establish correlations between oxidative/antioxidative markers and FM clinical parameters.

Methods

Study subjects

The research has been carried out in accordance with the Declaration of Helsinki (2008) of the World Medical Association. The study was approved by the Ethics Committee of the University of Jaén (Spain), and all subjects provided written informed consent. Eighty-two female patients with FM from AFIXA (Association of Fibromyalgia of Jaén, Spain) were interviewed for the study. Twenty-five age-matched healthy women were recruited from the University of Jaén (Spain).

The inclusion criteria for the FM group comprised that all of the patients met the 1990 American College of Rheumatology (ACR) Criteria for classification of primary FM (28). Exclusion criteria for both groups included the presence of any other chronic disease (diabetes mellitus, hypertension, cancer, ischemic heart disease), pregnancy, lactation, and grade II obesity (body mass index (BMI) ≥ 35 kg/m²). Neither patients nor controls were using any medicine that affects the antioxidative status. None of the participants were under the treatment of corticosteroids, oestrogens, analgesics or anti-inflammatory drugs, and were only included if they had stopped using them at least 2 months before the study. None was consuming alcohol, and all of them were non-

smokers. All the participants were sedentary living women.

The demographic and clinical data were obtained from patient interview and questionnaires. The same specialist carried out all the measurements and tests throughout the study. In FM patients, functional capacity in daily living activities was evaluated by Spanish version of Fibromyalgia Impact Questionnaire (FIQ) (29), and musculoskeletal pain was scored according to Visual Analogue Scale (VAS) (10 cm). The physical (Physical Component Summary, PCS-12) and mental (Mental Component Summary, MCS-12) health status of patients and controls was assessed by Spanish version of SF-12 Health Survey (30). The score ranges from 0 to 100 with lower values reflecting worse health status.

Blood collection and preparation of blood samples

Venous blood was taken in the early morning after an overnight fast from the antecubital vein into two EDTA and one EDTA-free tubes (total blood volume: 23.5 mL). Blood was collected at the same time of the day to prevent the daily variations of the level of antioxidants (31). The antioxidant activities (superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase) and 8-hydroxy-2'-deoxyguanosine were determined in lymphocytes. Separation of lymphocytes from whole blood (1 EDTA tube) was performed following the method of Boyum (32) through differential centrifugation using Histopaque-1077 (Sigma-Aldrich). Lymphocytes were stored at -80°C until used. Concerning the EDTA-free tube, blood was allowed to clot for 30 min at room temperature. One EDTA and one EDTA-free tubes were then centrifuged at 3500 rpm for 5 min at 4°C to obtain plasma and serum samples, respectively. Antioxidant compounds (copper, iron, bilirubin, uric acid, albumin, zinc, transferrin, ferritin, ceruloplasmin) were measured in serum samples following blood collection. For oxidative and antioxidative determinations (total antioxidant capacity (TAC), lipid peroxidation (TBARS), protein carbonyl content), plasma samples were stored at -80°C until used.

*Determination of the oxidative status**- Thiobarbituric acid reactive substances*

The measurement of thiobarbituric acid reactive substances (TBARS) is a good indicator of lipid peroxidation, a major marker of oxidative stress (33). TBARS was determined spectrophotometrically in plasma samples following the manufacturer's recommendations (TBARS Assay Kit, OXItek, Catalog. 0801192). The lipid peroxidation level was expressed as nmol of malondialdehyde (MDA) formed per mL.

- Protein carbonyl content

The most general indicator marker of protein oxidation is protein carbonyl content (19). The protocol was performed in plasma samples from FM patients and controls following the manufacturer's instructions (Protein Carbonyl Assay Kit, Cayman, item no. 10005020).

- 8-hydroxy-2'-deoxyguanosine

8-hydroxy-2'-deoxyguanosine (8-oxo-dG) has become a frequently used biomarker of oxidative DNA damage and oxidative stress (20). Nuclear DNA was isolated and digested following the method used by Espinosa *et al.* (34). The separation of 8-oxo-dG was performed by high performance liquid chromatography (HPLC), also following the Espinosa method (34). The amount of 8-oxo-dG and deoxyguanosine (dG) in the DNA digest was measured by electrochemical and UV absorbance detection, respectively (35).

*Determination of the antioxidative status**- Total antioxidant capacity*

The total antioxidant capacity (TAC) of plasma samples from patients with FM and healthy controls was performed following the manufacturer's instructions (Antioxidant Assay Kit, Cayman, item no. 709001).

- Antioxidant compounds

The antioxidant compounds were measured in serum samples from FM patients and volunteers during routine blood sampling for biochemical analyses. The analysers used to determine these compounds were the Olympus AU5400 (Beckman Coulter) (copper,

iron, bilirubin, uric acid, albumin, zinc, transferrin, ferritin) and the BN II System (Siemens Healthcare Diagnostics) (ceruloplasmin).

- Antioxidant activities

Superoxide dismutase: The activity of superoxide dismutase (SOD) was determined spectrophotometrically in patients with FM and controls following the method of Nebot *et al.* (36). Total protein content was estimated by the method of Markwell *et al.* (37), a modification of the colorimetric method of Lowry *et al.* (38). SOD activity was expressed relative to protein content as units per milligram of protein (U/mg protein).

Glutathione peroxidase: The glutathione peroxidase (GPx) activity was measured in lymphocytes from FM patients and volunteers according to the manufacturer's recommendations (Glutathione Peroxidase Assay Kit, Cayman, item no. 703102). GPx activity was normalised to protein content and expressed as units per gram of protein (U/g protein).

Catalase: The activity of catalase was determined spectrophotometrically in FM patients and controls following the manufacturer's instructions (BIOXYTECH Catalase-520, OxisResearch, Catalogue no. 21042). Catalase activity was referred to total protein concentration and expressed as units per gram of protein (U/g protein).

Statistical analysis

Calculations of sample size were performed using Ene 3.0 (GlaxoSmithKline SA, Madrid, Spain). Data for continuous variables were expressed as mean \pm standard deviation (SD). Management and data analysis were performed using the statistical package SPSS for Windows version 19.0 (SPSS Inc, Chicago, IL, USA). Kolmogorov-Smirnov test (α -value=0.05) and Levene test (α -value=0.05) were performed to test normality and homoscedasticity respectively. Data which followed a normal distribution and the principle of homoscedasticity of variances were tested by an unpaired Stu-

dent's *t*-test to compare differences between means. The degree of statistical significance in data which did not follow a normal distribution or the principle of homoscedasticity (SOD, ceruloplasmin, PCS-12, MCS-12) was established by applying the Mann-Whitney U-test. To assess the relation between continuous variables (oxidative/antioxidative stress markers and FM clinical parameters), Pearson correlation was used. The level of statistical significance was set at $p < 0.05$.

Results*Study subjects*

From the 82 patients with FM interviewed, 45 (mean (SD) age of 52.2 (7.5) years) met the inclusion criteria. Also 25 healthy age-matched controls (mean (SD) age of 49.6 (7.9) years) participated in the study. The demographic and clinical data of patients and controls are shown in Table I. The physical (PCS-12) and mental (MCS-12) health status of FM patients, assessed by SF-12 Health Survey, was significantly lower as compared to volunteers ($p < 0.001$, $p = 0.033$, respectively).

Determination of the oxidative status

The thiobarbituric acid reactive substances (TBARS) level remained unaltered in FM patients vs. healthy volunteers ($p = 0.766$). On the other hand, the protein carbonyl content and 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) level of patients with FM significantly rose in comparison to the control group ($p = 0.022$, $p < 0.001$, respectively) (Table II).

Determination of the antioxidative status

The total antioxidant capacity (TAC) (Table III) of patients with FM was significantly lower than in controls ($p = 0.023$). Alternatively, while some antioxidant compounds such as zinc decreased in FM patients vs. healthy controls ($p < 0.001$), copper ($p < 0.001$) and ceruloplasmin ($p < 0.001$) increased. Other antioxidant compounds, including bilirubin ($p = 0.719$), uric acid ($p = 0.543$), iron ($p = 0.917$), transferrin ($p = 0.486$), ferritin ($p = 0.261$) and albumin ($p = 0.744$), remained unaltered in

Table I. Demographic and clinical characteristics of fibromyalgia (FM) patients and healthy controls.

	Controls	FM patients	95% CI	p-value
Age (year)	49.6 ± 7.9	52.2 ± 7.5	- 8.96, 3.76	0.414
Gender (Female/Male)	25/0	45/0	-	-
FIQ score	-	61.39 ± 13.13	-	-
VAS score	-	6.43 ± 1.84	-	-
PCS-12 score	54.43 ± 4.18	33.34 ± 7.21	17.40, 24.78	<0.001
MCS-12 score	51.26 ± 3.82	40.74 ± 12.57	5.39, 15.62	0.033

Data are expressed as mean ± standard deviation, except for gender.
95% CI: 95% confidence interval; FIQ: Fibromyalgia Impact Questionnaire; VAS: visual analogue scale; PCS-12: physical health status; MCS-12: mental health status.

Table II. Oxidative status in fibromyalgia (FM) patients and healthy controls.

	Controls (n=25)	FM patients (n=45)	95% CI	p-value
TBARS (nmol MDA/mL)	15.54 ± 3.08	16.54 ± 3.84	-7.77, 5.77	0.766
Protein carbonyl content (nmol/mL)	13.77 ± 3.02	19.94 ± 4.09	-11.41, - 0.93	0.022
8-oxo-dG (8-oxo-dG/10 ⁶ dG)	3.72 ± 0.47	4.74 ± 0.80	-1.5, - 0.49	<0.001

Data are expressed as mean ± standard deviation.
95% CI: 95% confidence interval; TBARS: Thiobarbituric acid reactive substances; MDA: malondialdehyde; 8-oxo-dG: 8-hydroxy-2'-deoxyguanosine.

Table III. Antioxidative status in fibromyalgia (FM) patients and healthy controls.

	Controls (n=25)	FM patients (n=45)	95% CI	p-value
TAC (mmol/L)	244.62 ± 8.98	235.79 ± 10.95	1.28, 16.37	0.023
Uric acid (µg/dL)	3.73 ± 0.50	3.59 ± 0.72	- 0.31, 0.59	0.543
Bilirubin (µg/dL)	0.55 ± 0.19	0.57 ± 0.17	-0.14, 0.10	0.719
Copper (mg/dL)	83.55 ± 9.20	105.99 ± 17.03	-32.87, -12.00	<0.001
Zinc (ng/mL)	106.8 ± 22.41	66.48 ± 18.82	26.11, 54.51	<0.001
Iron (mg/dL)	83 ± 30.07	81.82 ± 34.64	-21.45, 23.79	0.917
Ferritin (g/dL)	57.42 ± 17.01	52.33 ± 15.07	-9.88, 20.07	0.486
Transferrin (mg/L)	267.09 ± 35.41	284.45 ± 45.78	- 48.16, 13.44	0.261
Albumin (µg/dL)	4.35 ± 0.26	4.38 ± 0.24	-0.19, 0.14	0.744
Ceruloplasmin (mg/dL)	24.35 ± 2.20	30.14 ± 5.14	-7.95, - 3.60	<0.001

Data are expressed as mean ± standard deviation.
95% CI: 95% confidence interval; TAC: total antioxidant capacity.

these patients in comparison to control group (Table III).

We found a significant decrease in the enzymatic activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (Table IV) in the FM group when compared to volunteers ($p < 0.001$, $p = 0.013$, $p = 0.004$, respectively).

Correlation between oxidative/antioxidative markers and clinical findings in FM patients

A significant positive correlation was observed in patients with FM between lipid peroxidation and impact of FM

on daily life (FIQ) ($p = 0.032$). Oxidative DNA damage negatively correlated with mental health status (MCS12) ($p = 0.039$), and a positive correlation between zinc and physical health status (PCS12) was found ($p = 0.017$) in these patients (Table V).

A number of significant correlations were also established between oxidative and antioxidative markers in FM patients. TBARS level significantly correlated with zinc ($r = -0.387$, $p = 0.022$). 8-oxo-dG levels showed a significant negative correlation with TAC ($r = -0.532$, $p = 0.001$), SOD ($r = -0.463$, $p = 0.006$), and catalase ($r =$

0.430 , $p = 0.011$). TAC positively correlated with SOD ($r = 0.750$, $p < 0.001$), catalase ($r = 0.923$, $p < 0.001$), and albumin ($r = 0.347$, $p = 0.044$). SOD showed a significant correlation with catalase ($r = 0.633$, $p < 0.001$), and albumin ($r = 0.440$, $p = 0.009$). Additionally, bilirubin correlated with iron ($r = 0.423$, $p = 0.011$), copper with ceruloplasmin ($r = 0.966$, $p < 0.001$), and iron with albumin ($r = 0.363$, $p = 0.032$).

Discussion

In recent years, the oxidant/antioxidant balance and its effects on the organism have gained much attention. Under normal circumstances, there is an appropriate balance between pro-oxidants and antioxidants. Cellular oxidative stress becomes manifest when excessive production of ROS overwhelm the antioxidant defense system in cells. Oxidative stress have been proposed to be implicated in the pathophysiology of fibromyalgia (FM), a poorly understood disease causing pain, stiffness, and tenderness of the muscles, tendons, and joints. The unavailability of specific anatomic, histological, or molecular disease markers complicates the diagnosis. The present work has been addressed to perform a complete and exhaustive study of the oxidative and antioxidative status in patients with FM and controls, as well as the relationship with FM clinical and functional parameters. We did not find another report in the literature investigating so many parameters of oxidative stress in the same FM patients.

We have examined the oxidative status of patients with FM and healthy volunteers, finding a significant increase in protein peroxidation and oxidative DNA damage, indicating that FM patients are exposed to oxidative stress. In agreement, increased levels of protein carbonyls were shown in female patients with FM *versus* controls (5). Oxidative stress is known to accelerate the generation of advanced glycation end-products (AGEs). AGE modification of proteins leads to high resistance to proteolytic digestion of such proteins. AGEs are also able to stimulate different types of cells by activating NF-κB, resulting in augmented expression

Table IV. Antioxidant enzyme activities in fibromyalgia (FM) patients and healthy controls.

	Controls (n=25)	FM patients (n=45)	95% CI	p-value
SOD (U/mg protein)	7.96 ± 1.19	4.72 ± 0.46	2.75, 3.79	<0.001
GPx (U/g protein)	50.63 ± 4.83	45.65 ± 5.71	1.11, 8.84	0.013
Catalase (U/g protein)	230.77 ± 8.47	217.14 ± 14.06	4.54, 22.70	0.004

Data are expressed as mean ± standard deviation.

95% CI: 95% confidence interval; SOD: superoxide dismutase; GPx: glutathione peroxidase.

of cytokines, adhesion molecules, and growth factors. Both mechanisms have been reported to contribute to the development and spreading of pain in patients with FM (39). To our knowledge, this is the first work that determines the level of 8-hydroxy-2'-deoxyguanosine (8-oxo-dG), a marker of oxidative damage to DNA, in patients with FM. Our findings revealed increased oxidative DNA damage in these patients, showing the role played by the oxidative pathway in the pathophysiology of FM. In this regard, studies in patients with chronic fatigue syndrome (CFS) have reported higher levels of 8-oxo-dG than in controls in muscle (14) and urine (40). The frequency of appearance of CFS in FM patients is about 20–70%, and the symptoms of these two diseases are similar. These results together imply increased ROS production in patients with FM, which may be implicated, at least in part, in the development of this disorder. In fact, an increase in free radicals can alter the functional capacity of the muscle, resulting in fatigability (41), a disabling symptom in FM

patients. Moreover, oxidative stress has been linked with disorganization of Z bands and abnormalities in number and shape of mitochondria in the muscles, as well as with damage to the sarcolemma resulting in muscles stiffness and pain (42). Our results also showed that TBARS level, marker of lipid peroxidation, did not change in FM patients when compared to healthy volunteers. In accord, Eisinger did not find differences in malondialdehyde levels between FM patients and control group (5, 43). On the contrary, several works have reported increased levels of lipid peroxidation in patients with FM (6, 7, 10, 44–46).

The antioxidative status was investigated through the measurement of the total antioxidant capacity (TAC) and the antioxidant enzyme activities and compounds. We found lower levels of TAC and zinc and higher levels of the antioxidant compounds copper and ceruloplasmin in FM patients as compared to healthy controls. In this regard, TAC was previously found diminished in women with FM in comparison to control group (9, 47), showing an alteration in the antioxidative status of these patients. Regarding the antioxidant compounds, zinc is critical for the formation and activity of many enzymes and cells that play a role in the maintenance of a healthy immune system. In agree with our findings, several studies have detected a decrease in zinc (12, 48) and an increase in copper (12) in patients with FM. In the light of these results is apparent that alteration in levels of trace elements such as zinc and copper may take part in the pathophysiology of FM. In this context, an association between serum zinc levels and number of tender points has been earlier reported in FM patients, proposing that zinc may be implicated in the

pathogenesis of this disorder (48). To our knowledge, this is the first work that investigates the ceruloplasmin level in patients with FM. Ceruloplasmin, the major copper-carrying protein, significantly increased in these patients, suggesting altered regulation of ceruloplasmin in females with FM. Such changes may lead to abnormal copper metabolism, which may have impact on the etiopathogenesis of FM.

We did not detect significant differences between FM patients and controls in the level of other antioxidant compounds such as bilirubin, albumin, uric acid, iron, transferrin, and ferritin. Similarly, unaltered levels of uric acid have been previously reported in urine from patients with FM *versus* normal volunteers (49). Regarding iron and iron stores, controversial results are found in the literature. On the one hand, FM patients reportedly showed a significantly lower level of serum ferritin (50) and iron in hair samples (51). On the other hand, iron, transferrin, and ferritin levels did not differ between FM and control groups (52). Our data suggest that neither iron nor iron stores seem to have a role in the etiopathology of this disorder.

It has been reported that altered activity of antioxidant enzymes may play a role in the etiopathogenesis of FM (8). Our results showed that antioxidant enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase were lower in FM patients *versus* healthy controls. In this context, debatable results have been reported concerning the antioxidant enzyme defense system in FM. In accord to our results, diminished SOD (6) and catalase were found in FM patients compared to volunteers (11, 45). Otherwise, no significant differences *versus* controls were showed in SOD in FM patients (7), neither in GPx in CFS subjects (53). The decreased activity of these antioxidant enzymes in patients with FM may alter the physiological levels of H₂O₂ and superoxide. Enhanced production of H₂O₂ has been reported to contribute to pain development in FM patients (54). The biological reactivity of superoxide is kept under the control of endogenous SOD. However, in pathological situa-

Table V. Correlation between oxidative/antioxidative markers and clinical/functional parameters in fibromyalgia (FM) patients.

	TBARS	
FIQ	r= 0.363	p = 0.032
	8-oxo-dG	
MCS-12	r= -0.355	p = 0.039
	Zinc	
PCS-12	r= 0.402	p = 0.017

r: Pearson correlation coefficient; TBARS: thiobarbituric acid reactive substances; 8-oxo-dG: 8-hydroxy-2'-deoxyguanosine; FIQ: Fibromyalgia Impact Questionnaire; PCS-12: physical health status; MCS-12: mental health status.

tions superoxide production can overwhelm the capacity of SOD to remove it, resulting in superoxide-mediated injury (55). In this context, superoxide has been proposed as a key player in pain (26).

If oxidative stress seems to be involved in the pathophysiology of FM, it is thought that it might affect FM symptoms. For this, we assessed correlations between oxidative/antioxidative markers and clinical manifestations of FM. In patients with FM, we determined severity of pain by visual analogue scale (VAS), and functional capacity by Fibromyalgia Impact Questionnaire (FIQ). The physical (PCS-12) and mental (MCS-12) health status of patients and volunteers was assessed by SF-12 Health Survey, showing significantly lower values in FM patients than in healthy controls. In FM patients, our results showed that oxidative DNA damage negatively correlated with mental health status, and a positive correlation was established between zinc and physical health status. The association between these markers and FM clinical parameters may suggest the involvement of oxidative stress in the decline of the health status of FM patients. Although the lipid peroxidation level did not change in FM patients when compared to healthy volunteers, our results in these patients showed a significant positive correlation between this oxidative stress marker and impact of FM on daily life. A number of studies have been published with contradicting results until now. In this sense, a significant correlation between lipid peroxidation and clinical parameters (VAS and FIQ scores) was observed in FM patients (44), as well as with headache measured by Headache Impact Test (45). In contrast, no correlations were previously found between lipid peroxidation, SOD, and pain (6, 7) and FIQ score (7). The reasons for such varied results may be the stage of the disease or the intrinsic peculiarities of each patient. We also found several significant correlations between oxidant and antioxidant markers in patients with FM. In this regard, the activity of the antioxidant enzymes SOD and catalase negatively correlated with oxidative DNA

damage, supporting that a decrease in the activity of these enzymes may increase oxidative stress in FM patients. The future of FM holds a great deal of interest, as its long-term effects on patients and its costs to society are becoming clearer. This study reveals an imbalance between oxidant and antioxidant levels in patients with FM. Our data provide evidence that diminished activity of endogenous antioxidant enzymes may lead to oxidative stress in these patients. Excessive production of ROS may cause oxidative damage to proteins and DNA, which might have an impact on the health status of FM patients, an important limiting factor of this disorder. These altered levels of oxidant and antioxidant markers may become useful biomarkers for FM, in order to aide in diagnosis and to guide therapy. These markers significantly correlate with clinical manifestations of FM, proposing that oxidative stress may be responsible, at least in part, for the development of FM. In this context, antioxidant strategies aimed to reduce oxidative stress may be indicated in these patients (56). However, future researches should evaluate the efficacy of such strategies in double blind and placebo controlled trials.

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