Malondialdehyde and sulfhydryl groups as biomarkers of oxidative stress in patients with systemic lupus erythematosus

Yenly G. Pérez[1], Lissett Caridad González Pérez[1], Rita de Cássia M. Netto[2], Domingos S. N. de Lima[3], Emerson S. Lima[4]

ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown origin associated with oxidative stress. The present study aimed to investigate the presence of oxidative stress in patients with newly diagnosed SLE. SLE patients (n = 36) and control subjects (n = 28) were enrolled in this study. Blood samples were used for malondialdehyde (MDA), sulfhydryl groups (SH) and uric acid determination. MDA levels (μmol/L) were higher in patients (3.9 ± 2.6) than in control subjects (1.6 ± 2.6). SH were significantly lower in SLE patients. The findings suggest that MDA can be a good marker of oxidative stress in SLE.

Keywords: oxidative stress, antioxidants, lupus erythematosus systemic, uric acid.

Systemic lupus erythematosus (SLE) is a chronic, autoimmune disorder characterized by a broad spectrum of clinical manifestations with multiple autoantibody production and complement-fixing immune complex deposition that result in tissue' damage.[1] Although the specific cause of SLE is unknown, several studies associate this disease to defective cellular and humoral immunity, probably influenced by genetic, environmental, and hormonal factors.[2,3]

Free radicals and other reactive oxygen/nitrogen/chlorine species are believed to contribute to the development of several chronic diseases by causing oxidative stress and oxidative damage. Diseases in which oxidative damage has been implicated include cancer, atherosclerosis, Alzheimer's disease, diabetes mellitus, and autoimmune diseases.[4–8]

Most clinical studies focus on the measurement of oxidative damage by using biomarkers – oxidants and antioxidants. Malondialdehyde (MDA), an oxidation product of lipoperoxidation, has been found elevated in various diseases.[9] Sulfhydryl (SH) groups (thiols) are considered the biggest and most frequently antioxidants in plasma.[10] Several experimental studies pointed to a qualitatively and quantitatively important role of uric acid as an antioxidant substance acting as a free radical scavenger and a chelator of transitional metal ions which are converted to poorly reactive forms.[11]

The purpose of this study was to determine the presence of oxidative stress in SLE patients by determining these biomarkers in blood samples. Parameters were correlated with disease activity and comorbidities; results were compared with normal subjects in the control group.

The study included 36 patients with SLE and 28 healthy volunteers (control) between the ages of 10 and 56. The diagnosis was based on at least four of the 11 diagnostic criteria established by the American College of Rheumatology (ACR).[12] All patients were in treatment and the disease activity was assessed by the Systemic Lupus Erythematosus Disease Active Index (SLEDAI). Disease was considered active when SLEDAI > 6.[13] The protocol of the study was approved by the Ethical Committee for Human Research from Universidade Federal do Amazonas (CAAE n. 0043.0.115.000.08). All patients and non-patients signed an informed consent form

Received on 09/07/2011. Approved on 05/08/2012. The authors declare no conflict of interest. Financial Support: FAPEAM, CNPq.
Faculdade de Ciências Farmacêuticas, Universidade Federal do Amazonas – UFAM.
1. Undergraduate Student of Medicine, Universidade Federal do Amazonas – UFAM
2. Student of the Post-Graduation Program, Genetics and Biochemistry Institute, Universidade Federal de Uberlândia – UFU
3. PhD; Reumatologist, Hospital Universitário Getúlio Vargas
4. PhD; Adjunct Professor, Faculdade de Ciências Farmacêuticas, UFAM
Correspondence to: Emerson S. Lima. Faculdade de Ciências Farmacêuticas. Universidade Federal do Amazonas. CEP: 69010-300. Manaus, AM, Brazil.
E-mail: eslima@ufam.edu.br

before taking part in the study and were submitted to a standardized questionnaire for demographic factors.

The venous blood collection (10 mL) from each participant was done with an evacuated tube system (BD Vacutainer® System) and centrifuged (800 g, 15 min). Serum was used for determining biochemical and immunological markers.

MDA was determined by High Performance Liquid Chromatography (HPLC); chromatograms were monitored at 532 nm and the sample concentration was determined in µmol/L.[14] Uric acid was measured using Cobas Mira® spectrophotometric analyzer (Roche Instruments Inc.), with commercially available kits (Labtest, Minas Gerais, Brazil). SH groups were determined by the Ellmans method, modified by Hu et al.[15] The results were expressed as means ± standard deviation (SD). Student's t-distribution was used to compare mean values. Pearson's and Spearman's correlations were applied to correlate the parameters with SLEDAI. $P < 0.05$ was considered to be statistically significant.

General and demographic characteristics of SLE patients and healthy controls are presented in Table 1. There was also no difference between duration, criteria number, and activity of the disease and oxidative stress ($P > 0.05$).

Lupus is characterized by direct aggression of autoantibodies and complement-fixing immune complex deposition that result in tissues' damage associated to oxidative stress.[16] Waszczykowska et al.,[17] suggested that intracellular free radicals are capable of inducing cytokine synthesis that participate and modulate inflammatory responses with the creation of superoxide radicals.

Oxidative stress, measured by MDA levels, was found increased in 78.9% (n = 30) of SLE patients, while only 21.1% (n = 8) of normal controls presented that increase (OR = 12.5; 95% CI 3.7–41.5). As shown in Table 2, MDA levels were found to be significantly increased in SLE patients compared to normal controls. No significant difference was found between MDA levels and the duration of the disease or comorbidities. Increased level of MDA in the serum[18] and in the erythrocytes[19] was reported in SLE patients. Wang et al.[20] and Shah et al.[21] associated stronger oxidative stress response with higher SLEDAI scores, similar to the previous report of Tewthanom et al.[18] However, we have not identified, in our study, the association of MDA or SH levels with SLEDAI scores. The high levels of MDA in SLE patients indicate that the lipid cell membrane was attacked and that MDA can be a good marker of oxidative stress in this disease.

Table 1
General and demographic characteristics of systemic lupus erythematosus patients and healthy controls

General data	SLE (n = 36)	Control (n = 28)
Age	28.2 ± 13	27.9 ± 9.9
Gender (female)	33 (91.6%)	28 (100%)
ACR number	5.3 ± 1.1	NA
DT (month)	5.9 ± 3.5	NA
SLEDAI number	10.3 ± 6.6	NA

SLE: systemic lupus erythematosus; ACR: American College of Rheumatology; DT: disease time; NA: not applicable.
Values are expressed as mean ± SD.

Table 2
Comparison between oxidant and antioxidant parameters in patients with systemic lupus erythematosus and healthy controls

Mean	SLE (n = 36)	Control (n = 28)	P*
MDA (µmol/L)	3.9 ± 2.6	1.6 ± 2.6	0.001
SH group (µmol/L)	260.2 ± 182.7	339.4 ± 104.3	0.04
Uric acid (mg/dL)	4.1 ± 1.5	3.8 ± 0.9	0.48

SLE: systemic lupus erythematosus; MDA: malondialdehyde; SH: sulfhydryl.
Values are expressed as mean ± SD and the differences were considered significant when $P < 0.05$.

There was no significant change in serum levels of uric acid in SLE patients compared to normal controls (4.1 ± 1.5 and 3.8 ± 0.9 mg/dL, respectively). No correlation was found between the serum levels of this compound and disease activity. Deminice et al.[22] associated uric acid as an oxidative stress biomarker response to an acute session of hypertrophy-resistance traditional interval training and circuit training. Ikeda et al.[23], however, could not make the same association when oxidative stress was observed in patients with progressive amyotrophic lateral sclerosis. Although uric acid is consider an important antioxidant and its serum levels were expected to be lower in SLE patients than in normal controls, our study could not associate this substance as a secure biomarker of oxidative stress either.

Morgan et al.[24] showed that markers of protein oxidation correlate with worsening disease status in SLE. In our study, SH group levels were found to be significantly decreased in SLE patients compared to normal controls (260.2 ± 182.7 *versus* 339.4 ± 104.3 µmol/L), similar to the report of Zhang et al.[25] This supports the role of oxidative stress in the pathogenesis of SLE.

We concluded that SLE patients present increases in oxidative stress. However, this response is not correlated to the disease activity or its duration. MDA and SH group levels can be used as biomarkers to measure oxidative stress in SLE patients, whereas uric acid cannot be used for the same purpose. Further studies on oxidative stress and SLE are still necessary to improve our understanding of the disease pathogenesis.

REFERENCES

1. Karanam SA, Dharmavarapu PK, D Souza R, Upadhya S, Kumar V, Kedage V *et al*. Lúpus eritematoso sistêmico. In: Goldman L, Ausiello, Cecil D (eds.). *Tratado de medicina interna*. 22.ed. Rio de Janeiro: Elsevier; 2005, p.1937–47.
2. Kumar V, Abbas AK, Fausto N. Adaptação, dano e morte celular. In: Kumar V, Abbas AK, Fausto N, Robbins & Cotran (eds.). *Bases patológicas das doenças*. 7.ed. Rio de Janeiro: Elsevier; 2005, p.17–9.
3. Nery FG, Borba EF, Neto FL. Influence of the psychosocial stress on systemic lupus erythematosus. Rev Bras Reumatol 2004; 44(5):355–61.
4. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006; 160(1):1–40.
5. Bonomini F, Tengattini S, Fabiano A, Bianchi R, Rezzani R. Atherosclerosis and oxidative stress. Histol Histopathol 2008; 23(3):381–90.
6. Zawia NH, Lahiri DK, Cardozo-Pelaez F. Epigenetics, oxidative stress, and Alzheimer disease. Free Radic Biol Med 2009; 46(9):1241–9.
7. Chang YC, Chuang, LM. The role of oxidative stress in the pathogenesis of type 2 diabetes: from molecular mechanism to clinical implication. Am J Transl Res 2010; 2(3):316–31.
8. Filippin LI, Vercelino R, Marroni NP, Xavier RM. Redox influence on the inflammatory response in rheumatoid arthritis. Rev Bras Reumatol 2008; 48(1):17–24.
9. Amara A, Constans J, Chaugier C, Sebban A, Dobourg L, Peuchant E *et al*. Autoantibodies to malondialdehyde-modified epitope in connective tissue diseases and vasculitides. Clin Exp Immunol 1995; 101:233–8.
10. Kolagal V, Karanam SA, Dharmavarapu PK, D´souza R, Upadhya S, Kumar V *et al*. Determination of oxidative stress markers and their importance in early diagnosis of uremia-related complications. Indian J Nephrology 2009; 19(1):8–12.
11. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. Curr Pharm Des 2005; 11(32):4145–51.
12. Surita FGC, Cecatti JG, Barini R, Parpinelli MA, Silva JLC. Lúpus e gravidez. Rev Bras de Ginec e Obstetrícia 1997; 19(6):413–7.
13. Morgan PE, Sturgess AD, Davies MJ. Increased levels of serum protein oxidation and correlation with disease activity in SLE. Arthritis Rheum 2005; 52(7):2069 –79.
14. Fukunaga K, Takama K, Suzuki T. High performance liquid chomatography determination of plasma malondialdehyde level without a solvent extraction procedure. Anal Biochem 1995; 230(1):20–3.
15. Hu ML, Louie S, Cross CE, Motchnik P, Halliwell B. Antioxidant protection against hypochlorous acid in human plasma. J Lab Clin Med 1993; 121(2):197–8.
16. Ferguson JD. Stress that causes palpitations: oxidative stress and atrial fibrillation. Heart Rhythm 2009; 6(7):941–2.
17. Waszczykowska E, Robak E, Wozniacka A, Narbutt J, Torzecka JD, Sysa-Jedrzejowska A. Estimation of SLE activity based on the serum level of chosen cytokines and superoxide radical generation. Mediators of Inflammation 1999; 8(2):93–100.
18. Tewthanom K, Janwityanuchit S, Totemchockchyakarn K, Panomvana D. Correlation of lipid peroxidation and glutathione levels with severity of systemic lupus erythematosus: a pilot study from single center. J Pharm Pharm Sci 2008; 11:30–4.
19. Turgay M, Durak I, Erten S, Ertugrul E, Devrim E, Avci A *et al*. Oxidative stress and antioxidant parameters in a Turkish group of patients with active and inactive systemic lupus erythematosus. APLAR J Rheumatol 2007; 10:101–6.
20. Wang G, Pierangeli SS, Papalardo E, Ansari GA, Khan MF. Markers of oxidative and nitrosative stress in systemic lupus erythematosus: correlation with disease activity. Arthritis Rheum 2010; 62(7):2064–72.
21. Shah D, Kiran R, Wanchu A, Bhatnagar A. Oxidative stress in systemic lupus erythematosus: Relationship to Th1 cytokine and disease activity. Immunol Letters 2010; 129:7–12.
22. Deminice R, Sicchieri T, Mialich MS, Milani F, Ovidio PP, Jordan AA. Oxidative stress biomarker response to an acute session of hypertrophy-resistance traditional interval training and circuit training. J Strenght Cond Res 2011; 25(3):798–804.
23. Ikeda K, Kawabe K, Iwasaki Y. Do serum acid reflect oxidative stress in the progression of ALS? J Neurol Sci 2009; 287(1–2):294.
24. Morgan PE, Sturgess AD, Davies MJ. Evidence for chronically elevated serum protein oxidation in systemic lupus erythematosus patients. Free Radic Res 2009; 43:117–27.
25. Zhang Q, Ye DQ, Chen GP, Zheng Y. Oxidative protein damage and antioxidant status in systemic lupus. Eryth Clin Exp Dermatol 2009; 35:287–94.