MARCELL ALYSSON BATISTI LOZOVOY

RESISTÊNCIA À INSULINA E HIPERTENSÃO ARTERIAL EM PACIENTES COM LÚPUS ERITEMATOSO SISTÊMICO:

AVALIAÇÃO DA RESPOSTA IMUNE Th1/Th2/Th17, DO METABOLISMO DE FERRO, DO ÓXIDO NÍTRICO E DO ESTRESSE OXIDATIVO.

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Dissertação apresentada ao Programa de Pós-Graduação em Patologia Experimental da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Doutor em Patologia Experimental.

Orientador: Prof. Dr. Isaias Dichi.

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2012

Catalogação elaborada pela Divisão de Processos Técnicos da Biblioteca Central da Universidade Estadual de Londrina

Dados Internacionais de Catalogação-na-Publicação (CIP)

L925r Lozovoy, Marcell Alysson Batisti.
Resistência à insulina e hipertensão arterial em pacientes com lúpus eritematoso sistêmico: avaliação da resposta imune Th1/Th2/Th17, do metabolismo de ferro, do óxido nítrico e do estresse oxidativo. / Marcell Alysson Batisti Lozovoy – Londrina, 2012.
146 f. : il.

Orientador: Isaias Dichi.
Dissertação (Doutorado em Patologia Experimental) – Universidade Estadual de Londrina, Centro de Ciências Biológicas, Programa de Pós-Graduação em Patologia Experimental, 2012.
Inclui bibliografia.

1. Lúpus eritematoso sistêmico – Teses. 2. Resistência à insulina. – Teses. 3. Hipertensão arterial – Teses. 4. Estresse oxidativo. – Teses. I. Dichi, Isaias. II. Universidade Estadual de Londrina. Centro de Ciências Biologicas. Programa de Pós-Graduação em Patologia Experimental. III. Título.

616-092

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Londrima, 14 de novembro de 2012.

Esta tese eu dedico a minha família,

à minha esposa Andréa e minhas filhas

Maria Eduarda e Maria Luíza.

AGRADECIMENTOS

Agradeço primeiramente a Deus, pela vida que nos permite viver, nos dando força e sabedoria para sempre podermos crescer cientificamente e espiritualmente.

Ao meu orientador Prof. Dr. Isaias Dichi por todo apoio para realização deste trabalho.

Ao Prof. Dr. Rubens Cecchini, coordenador do programa de doutorado em patologia experimental, pela sua enorme contribuição científica, disponibilidade e gentileza, essenciais para a realização deste trabalho.

À Profa. Dra. Andréa Name Colado Simão que através de seu conhecimento, sabedoria e compreensão possibilitaram a construção e execução deste trabalho.

Às docentes Profa. Dra. Edna Maria Vissoci Reiche e Profa. Msc. Helena Kaminami Morimoto pela contribuição na viabilização e realização de vários parâmetros laboratoriais deste trabalho.

Aos Professores do Programa de Pós-Graduação em Patologia Experimental pelos ensinamentos fundamentais para realização deste trabalho.

À Dra. Carolina Panis por realizar a determinação dos metabólitos do óxido nítrico.

Aos estagiários, Eloisa da Silva e Diego Lima Petenucci, acadêmicos da iniciação científica e à mestre Sayonara Rangel Oliveira por seu tempo e dedicação em realizar as técnicas de estresse oxidativo.

À médica Tatiana Mayumi Veiga Iryioda do Ambulatório de Reumatologia do Hospital Universitário Norte do Paraná, que avaliou clinicamente os pacientes participantes deste projeto.

Aos meus amigos e familiares, pela compreensão dos momentos juntos que tivemos que abrir mão nestes últimos tempos, pelo apoio e carinho.

LOZOVOY, Marcell Alysson Batisti. **Resistência à insulina e hipertensão arterial em pacientes com lúpus eritematoso sistêmico:** avaliação da resposta imune Th1/Th2/Th17, do metabolismo de ferro, do óxido nítrico e do estresse oxidativo. 2012. 146f. Dissertação (Doutorado em Patologia Experimental) – Universidade Estadual de Londrina, Londrina, 2012.

RESUMO

O Lúpus Eritematoso Sistêmico (LES) é uma doença autoimune multissistêmica caracterizada pelo envolvimento de vários órgãos e por altos títulos de anticorpos contra antígenos nucleares e citoplasmáticos. O LES tem um forte componente inflamatório crônico e, conseqüentemente, ocorre aumento na produção de espécies reativas de oxigênio (ROS) e espécies reativas de nitrogênio (RNS). O estresse oxidativo pode contribuir para a disfunção das células imunes, a produção de autoantígeno e reatividade de autoanticorpos no LES. Na doença autoimune, as proteínas modificadas oxidativamente podem gerar neoepitopos desencadeando a autoimunidade. Inflamação crônica sistêmica e estresse oxidativo têm sido propostos ter um papel proeminente na patogênese da resistência à insulina (RI). Os pacientes com LES têm maior RI e maior prevalência de síndrome metabólica (SM) do que a população em geral. No entanto, até a presente data, a RI e a prevalência de SM em pacientes com LES ativo e inativo não foram investigadas. A hipertensão é um importante fator de risco para doença cardiovascular e renal e é altamente prevalente em mulheres com LES. A hipertensão arterial resulta de fatores ambientais, genéticos, imunológicos e das interações entre esses fatores. A importância dos rins no controle da pressão arterial e da patogênese da hipertensão é bem compreendida e estima-se que quase 50% dos pacientes com LES sejam afetadas pela glomerulonefrite, mesmo isto não sendo um prerequisito fundamental para desenvoler hipertensão. No entanto, o LES é um fator de risco para a hipertensão arterial e pode ocorrer independentemente da presença de nefrite. Tem sido sugerido que o processo inflamatório crônico e o predomínio da linhagem de citocinas de células T "helper" 1 (Th1) como interferon gama (IFN-γ), fator de necrose tumoral alfa (TNF-α), entre outros sobre a linhagem de citocinas Th2 como a interleucina 4 (IL-4) e interleucina 10 (IL-10) podem contribuir para o aumento da pressão arterial. Os níveis séricos de ferritina e ferro livre também tem sido implicados na patogênese de muitas condições, incluindo doenças autoimunes e resistência à insulina. Ferritina elevada pode interferir com a extração hepática da insulina levando à hiperinsulinemia periférica e resistência à insulina. O objetivo principal desta tese foi avaliar a freqüência de resistência à insulina e hipertensão e os possíveis fatores associados a estas situações em pacientes com LES sem insuficiência renal. Os resultados obtidos foram apresentados e discutidos em 3 artigos científicos cujo delineamento, metodologia e resultados são descritos a seguir.

Artigo 1: Os objetivos deste estudo foram verificar a prevalência de resistência à insulina e síndrome metabólica em pacientes brasileiros com LES e analisar se a atividade da doença interfere com as condições acima mencionadas. O estudo incluiu 130 controles e 74 pacientes com LES. Pacientes com LES foram divididos em doença ativa (36 pacientes) e doença não-ativa (38 pacientes). A prevalência de resistência à insulina, avaliada por HOMA-IR (*Homeostasis Model Assessment - Insulin Resistance*) foi verificada em 38 (51,35%) pacientes com LES e 38 (29,23%) no grupo controle (p = 0,0017, OR: 2,556, IC 95%: 1,413-4,621), enquanto a prevalência de síndrome metabólica, avaliada pelo ATP-III (*Adult Treatment Panel III*) foi observada em 25 (33,78%) em pacientes com LES e 17 (13,08%) no grupo controle (p <0,0001, OR = 4,644, IC 95%: 2,644-9,625). A resistência à insulina foi

verificada em 23 (63,89%) pacientes com LES ativo e em 15 (39,47%) de pacientes com LES inativo (OR: 2,781, IC 95%: 1,568-4,932, p = 0,0004), enquanto que 15 (41,67%) pacientes com LES ativo preencheram os critérios para síndrome metabólica em comparação com 10 (26,32%) com LES inativo (OR: 2,061, IC 95%: 1,133-3,748, p = 0,0169). O índice de massa corporal e a corticoterapia foram significativamente superiores no grupo LES ativo. Este estudo reforça o maior risco de desenvolver resistência à insulina e síndrome metabólica em pacientes com LES, especialmente naqueles com doença ativa, e apóia o papel da resistência à insulina e uso de corticosteróides como as principais ligações entre a atividade da doença e síndrome metabólica.

Artigo 2: Os objetivos deste estudo foram investigar diversos fatores, que influenciam a hipertensão em pacientes com LES ativo (AT) e não-ativo (NAT) sem comprometimento renal. Foram selecionados 102 indivíduos saudáveis (grupo controle), 70 pacientes com LES NAT e 53 LES AT. Pacientes com LES AT tiveram maior probabilidade de apresentar hipertensão quando comparados aos controles ($p < 0,0001$, OR: 4,101, IC 95%: 2,22-7,58) e também quando comparado com pacientes com LES NAT ($p = 0,0016$, OR: 2,510 IC 95%: 1,408-4,473). As citocinas plasmáticas foram avaliadas por metodologia de ELISA (*Enzyme-Linked Immunosorbent Assay*). Pacientes com LES apresentaram menores níveis plasmáticos de TNF-α e IL-4 ($p < 0,05$ e $< 0,0001$, respectivamente) e níveis significativamente superiores de interleucina 6 (IL-6), IL-10, interleucina 17 (IL-17), e IFN-γ ($p < 0,0001$, $< 0,01$, $< 0,0001$ e $< 0,05$, respectivamente) quando comparados ao grupo controle. Pacientes com LES apresentaram maior relação IFN-γ/IL-4 ($p < 0,01$) que o grupo controle, enquanto que em pacientes com LES AT apresentaram maior relação IL-12/IL-4 ($p < 0,05$) do que controles e pacientes com LES NAT. Pacientes com LES AT e NAT apresentaram níveis mais elevados de hidroperóxidos (avaliados por *Tert-butyl hydroperoxide-initiated chemiluminescence*-CL-LOOH) ($P < 0,05$ e $< 0,0001$, respectivamente) do que os controles, enquanto os níveis de oxidação de proteínas séricas (avaliadas por *Advanced Oxidation Protein Products*-AOPP) foram significativamente maiores em pacientes com LES AT em relação ao grupo controle e também em relação aos pacientes com LES NAT ($p < 0,01$ e $< 0,05$, respectivamente). A capacidade antioxidante total corrigida pelos níveis séricos de ácido úrico (TRAP / UA) (avaliados por quimiluminescência) foi significativamente menor no grupo LES do que no grupo controle ($p < 0,0001$). Os níveis séricos de metabólitos de óxido nítrico (avaliados pela metodologia da reação de Griess) foram significativamente mais elevados em pacientes com LES em relação ao grupo controles ($p < 0,0001$). O presente estudo mostrou que pacientes com LES AT têm maior probabilidade de desenvolver hipertensão arterial do que indivíduos controles e pacientes com LES NAT. Os principais fatores que influenciaram a maior freqüência de hipertensão arterial em pacientes com AT foram o aumento da relação Th1/Th2 proporção e do estresse oxidativo.

Artigo 3: Os objetivos deste estudo foram avaliar o estresse oxidativo e metabolismo do ferro em pacientes com LES com e sem resistência à insulina. Este estudo incluiu 236 indivíduos (125 controles e 111 pacientes com LES). Os pacientes com LES foram subdivididos em dois grupos: com ($n = 72$) ou sem ($n = 39$) a resistência à insulina. Pacientes com LES com resistência à insulina apresentaram maiores níveis de produtos avançados de oxidação protéica ($p = 0,030$), aumento da atividade da gama-glutamil transferase (GGT) ($p = 0,001$) e diminuição de agrupamentos sulfidrila de proteínas ($p = 0,0002$) e da capacidade antioxidante total corrigida pelos níveis de AU ($p = 0,04$) quando comparado com pacientes com LES sem resistência à insulina. No entanto, os pacientes com LES e resistência à insulina apresentaram menores níveis séricos de 8-isoprostanos ($p = 0,05$) e de proteínas carbonílicas ($p = 0,04$) quando comparado aos pacientes com LES sem resistência à insulina. Os níveis séricos de ferritina foram significativamente maiores em pacientes com LES ($p = 0,0006$) quando comparados aos controles e pacientes com LES com resistência à insulina apresentaram

maiores níveis de ferritina sérica (p = 0,01) em comparação com pacientes com LES sem resistência à insulina. Em pacientes com LES foi demonstrada uma correlação inversa entre a resistência à insulina e a capacidade antioxidante total (r = -0,2724, p = 0,0008) e a ferritina sérica foi positivamente correlacionada com os produtos avançados de oxidação protéica (r = 0,2870, p = 0,004). Este estudo demonstrou que a presença de resistência à insulina esteve diretamente associada ao aumento de estresse oxidativo em pacientes com LES e que o aumento de ferritina, o processo inflamatório e a hiperinsulinemia podem favorecer o desequilíbrio redox. O presente estudo reforça a necessidade de avaliar o estresse oxidativo por meio de vários parâmetros.

Palavras-chave: Lúpus eritematoso sistêmico. Resistência à insulina. Hipertensão arterial. Estresse oxidativo. Citocinas.

LOZOVOY, Marcell Alysson Batisti. **Insulin resistance and hypertension in patients with systemic lupus erythematosus:** evaluation of immune response Th1/Th2/Th17, iron metabolism, nitric oxide and oxidative stress. 146 p. Dissertation (Master´s degreen in Experimantal Pathology) – Universidade Estadual de Londrina, Londrina, 2012.

ABSTRACT

Systemic Lupus Erythematosus (SLE) is a autoimmune disease characterized by multisystem organ involvement and by high titers of autoantibodies against several nuclear and cytoplasmatic antigens. SLE has a strong inflammatory component and consequently chronic over-production of reactive oxygen species (ROS) and reactive nitrogen species occurs (RNS). Oxidative stress may contribute to immune-cell dysfunction, autoantigen production and autoantibody reactivity in SLE. In autoimmune disease, oxidatively modified proteins can generate neo-epitopes from self-proteins, causing aggressive autoimmune attack. Systemic chronic inflammation and oxidative stress has been proposed to have a prominent role in the pathogenesis of insulin resistance. Patients with SLE have higher insulin resistance and metabolic syndrome prevalence than the general population. However, to date, insulin resistance and metabolic syndrome prevalence in active and inactive disease have not been reported. Hypertension is a major risk factor for cardiovascular and renal diseases and is highly prevalent in women with SLE. Elevated blood pressure (BP) results from environmental, genetic and immunological factors, and from interactions among these factors. The importance of the kidneys in the long-term control of BP and the pathogenesis of hypertension are well understood and it is estimated that almost 50% of patients with SLE is affected by glomerulonephritis. However, SLE is a risk factor for hypertension in humans and can occur independently of nephritis. It has been suggested that chronic inflammatory process and higher Th1 cytokines profile such as interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α) and its predominance over Th2 cytokines profile such as interleukin 4 (IL-4) and interleukin 10 (IL-10) can contribute to increase DP. Ferritin and iron homeostasis have also been implicated in the pathogenesis of many conditions, including autoimmune disease and insulin resistance. Elevated ferritin may interfere with hepatic insulin extraction leading to peripheral hyperinsulinemia and insulin resistance. The main objective of this thesis was to evaluate the frequency of insulin resistance and hypertension and the possible factors associated with these situations in patients with SLE without renal impairment. The results were presented and discussed in 3 scientific articles whose design, methodology and results are described below.

Article 1: The objectives of this study were to verify the prevalence of insulin resistance and metabolic syndrome (MetS) in Brazilian patients with SLE and to analyze whether disease activity interferes with the aforementioned conditions. The study included 130 controls and 74 SLE patients. SLE patients were divided in active (36 patients) and inactive (38 patients) disease. Insulin resistance prevalence measured by HOMA-IR (Homeostasis Model Assessment - Insulin Resistance) was verified in 38 (51.35%) patients with SLE and in 38 (29.23%) controls (p=0.0017, OR: 2.556, IC 95%: 1.413-4.621), whereas MetS prevalence measured by ATP-II (Adult Treatment Panel III) was observed in 25 (33.78%) patients with SLE and in 17 (13.08%) controls (p<0.0001, OR=4.644, CI 95%: 2.644-9.625). Insulin resistance was verified in 23 (63.89%) patients with active SLE and in 15 (39.47%) patients with inactive SLE (OR: 2.781, IC 95%: 1.568-4.932, p=0.0004), whereas 15 (41.67%) patients with active SLE met the criteria for metabolic syndrome compared with 10 (26.32%) with inactive SLE (OR: 2.061, IC 95%: 1.133-3.748, p=0.0169). Body mass index and

corticosteroids use significantly increased in the active group. This study reinforces the higher risk for developing insulin resistance and metabolic syndrome in patients with SLE, especially in those with active disease, and support the role of insulin resistance and corticosteroid use as the main links between disease activity and metabolic syndrome.

Article 2: The objectives of this study were to investigate several factors, which influence hypertension in patients with active (AT) and non-active (NAT) systemic lupus erythematosus (SLE) without renal damage. This study enrolled 102 healthy individuals (control group), 70 NAT and 53 AT SLE patients without renal disease. SLE AT patients presented higher probability to develop hypertension when compared to controls ($p<0.0001$, OR:4.101, CI 95%: 2.22-7.58) and also when compared to NAT SLE patients ($p=0.0016$, OR: 2.510 CI 95%: 1.408-4.473). SLE patients showed lower TNF-α and IL-4 levels ($p < 0,05$ and $< 0,0001$, respectively) and higher interleukin 6 (IL-6), IL-10, interleukin 17 (IL-17), and IFN-γ levels ($p < 0,0001$, $< 0,01$, $< 0,0001$ and $< 0,05$, respectively) than the control group. SLE patients had higher IFN-γ/IL4 ratio ($p < 0,01$) than the control group, whereas AT SLE patients showed higher IL-12/IL-4 ratio ($p < 0,05$) than controls and NAT patients. SLE AT and NAT patients presented higher hydroperoxydes levels (measured by tert-butyl hydroperoxide-initiated chemiluminescence-CL-LOOH) ($P < 0,05$ and $< 0,0001$, respectively) than controls, whereas serum protein oxidation levels (measured by advanced oxidation protein products-AOPP) were significantly higher in AT SLE patients compared with the control group and also in relation to NAT SLE patients ($p < 0,01$ and $< 0,05$, respectively). Total radical-trapping antioxidant parameter corrected by uric acid (TRAP/UA) (measured by chemiluminescence) was significantly lower in NAT SLE than in the control group ($p < 0,0001$). Serum nitric oxide levels (measured by Griess reaction) were significantly higher in SLE patients in relation to the control group ($p < 0,0001$). The present study showed that patients with AT SLE had more probability to develop hypertension than control subjects and patients with NAT SLE. The main factors, which influenced the high frequency of hypertension in AT SLE patients were increased Th_1/Th_2 ratio and increased oxidative stress.

Article 3: The objectives of this study were to assess the oxidative stress and iron metabolism in SLE patients with and without insulin resistance (IR). This study included 236 subjects (125 controls and 111 SLE patients). Patients with SLE were divided in two groups: with (n=72) or without (n=39) IR. SLE patients with IR showed higher advanced oxidation protein products **(**AOPP) ($p = 0,030$), and gamma-glutamyltransferase levels ($p = 0,001$), and lower sulfhydryl groups of proteins ($p = 0.0002$) and total radical-trapping antioxidant parameter (TRAP) corrected by uric acid (UA) levels ($p = 0.04$) when compared to SLE patients without IR. However, SLE patients with IR presented lower serum 8-isoprostanes ($p = 0.05$) and carbonyl protein levels ($p = 0.04$) when compared to SLE patients without IR. Serum ferritin levels were significantly higher in SLE patients ($p = 0.0006$) when compared to controls and SLE patients with IR presented higher serum ferritin levels ($p = 0.01$) compared to SLE patients without IR. Patients with SLE showed that IR was inversely correlated to TRAP/UA ($r = -0.2724$, $p = 0.0008$) and serum ferritin was positively correlated with AOPP ($r = 0.2870$, $p = 0.004$). This study showed that IR increases oxidative stress in patients with SLE and that increased ferritin whatever its cause, the inflammatory process per se or hyperinsulinemia, can favor the redox process. The preset data reinforce the need of measuring oxidative stress with several methodologies with different assumptions.

Keywords: Systemic lupus erythematosus. Insulin resistance. Hypertension. Oxidative stress. Cytokine.

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LISTA DE SIGLAS E ABREVIATURAS

ANAs	Anticorpos antinucleares
Anti-dsDNA	Anticorpos anti-ácido desoxiribonucléico dupla fita
Anti-Sm	Anticorpo anti-Smith
Anti-RNP	Anticorpo anti-ribonucleoproteína-U1 RNP
Anti-SS-A	Anticorpo anti-Ro
Anti-SS-B	Anticorpo anti-La
ATPIII	"*Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults*"
C1q	Complemento 1 fração q
C1 r/s	Complemento 1 fração r/s
C2, C3 e C4	Componentes 2, 3 e 4 do Sistema Complemento
CAR	Colégio Americano de Reumatologia
CD	*Cluster of Differentiation*
CL-LOOH	Lipoperóxido
CYP	Citocromo P450
DAC	Doença arterial coronariana
DNA	Ácido desoxiribonucléico
ELISA	*Enzyme-Linked Immunosorbent Assay*
ENAs	Antígenos extraíveis do núcleo
Enos	Óxido nítrico sintase endotelial
EO	Estresse oxidativo
FAN	Fator antinúcleo
GLUT	Transportador de glicose
GPX	Glutationa peroxidase
GSH	Glutationa reduzida
H_2O_2	Peróxido de hidrogênio
HDL-C	Lipoproteínas de baixa densidade-colesterol
HLA	Antígeno leucocitário humano
HOMA-RI	Modelo de avaliação da homeostase-Resistência à insulina
HSP 70	Proteína do choque térmico 70kD
ICAM	Molécula de adesão intercelular 1
IFI	Imunofluorescência indireta

Ig	Imunoglobulina
IL-2, 6, 10, 17 e 21	Interleucinas-2, 6, 10, 17 e 21.
IMC	Índice de massa corporal
IFN-α	Interferon α
IFN-γ	Interferon γ
iNOS	Óxido nítrico sintase induzível
LDL-C	Lipoproteína de alta densidade-colesterol
LES	Lupus Eritematoso Sistêmico
MDA	Malondialdeído
NADPH	Fosfado de dinucleotídeo nicotinamida e adenina
NCEP	"*Executive Summary of the Third Report of the National Cholesterol Education Program"*
NFκB	Fator nuclear κB
NK	*Natural Killer*
NO	Óxido nítrico
NOx	Metabólitos do óxido nítrico
O_2	Oxigênio molecular
O_2	Ãnion superóxido
OMS	Organização Mundial da Saúde
PCR	Proteína C reativa
RI	Resistência à Insulina
RNA	Ácido ribonucléico
RNS	Espécies reativas de nitrogênio
RO	Radical alcooxil
ROO	Radical peroxil
ROS	Espécies reativas de oxigênio
SLEDAI	*Systemic Lupus Erythematosus Disease Activity Index*
SM	Síndrome Metabólica
SOD	Superóxido dismutase
SRA	Sistema renina-angiotensina
TAS	Estado antioxidante total
Tg	Triacilgliceróis
Th	Células T *helper* ou auxiliares
TNF	Fator de necrose tumoral

TRAP	Total radical-trapping antioxidant parameter. Capacidade antioxidante total.
TBARS	Substâncias reativas ao ácido tiobarbitúrico

SUMÁRIO

1 INTRODUÇÃO

1.1 LÚPUS ERITEMATOSO SISTÊMICO (LES)

Lúpus Eritematoso Sistêmico (LES) é uma doença autoimune, inflamatória e crônica, sendo seu desenvolvimento atribuído a fatores genéticos e ambientais em que há disfunção da resposta imune adquirida. No entanto, uma inadequada, ativa e sustentada resposta imune inata está implicada no início e nas conseqüências patogênicas da produção de autoanticorpos no LES (OATES, 2010).

O LES é caracterizado pela presença de autoanticorpos contra o núcleo celular, denominados anticorpos antinucleares (ANAs). Os ANAs podem estar direcionados contra as seguintes estruturas:: (1) anticorpos contra o ácido desoxirribonucleico dupla fita (anti-dsDNA); (2) anticorpos contra as proteínas histonas; (3) anticorpos contra proteínas ligadas ao ácido ribonucléico (anti-RNA); e (4) anticorpos contra antígenos nucleolares (KOTZIN; O´DELL, 1995; HAHN, 1998).

A presença destes anticorpos, principalmente o DNA e as proteínas histonas, geram reações locais e sistêmicas que acarretam comprometimento multissitêmico afetando principalmente articulações, pele, rins, pulmões, sistema nervoso, e outros órgãos do corpo. A doença pode variar de leve a grave, alternando períodos de atividade, caracterizada por manchas na face e pele, artralgia, febre, fadiga e períodos assintomáticos (URAMOTO et al., 1999).

1.2 EPIDEMIOLOGIA DO LÚPUS ERITEMATOSO SISTÊMICO

O LES está presente usualmente em mulheres na fase da pré-menopausa, entre 20 a 40 anos de idade, sendo o sexo feminino mais acometido que o masculino na proporção de 9:1. Também é mais comum em determinados grupos étnicos como, afro descendentes, asiáticos e hispânicos (URAMOTO et al., 1999). A freqüência da doença na população mundial é, em média, de aproximadamente 27,7/100mil (D´CRUZ et al., 2007), variando em torno de 15 a 120/100mil indivíduos (RUS; HOCHBERG, 2002), mas pode chegar a 400/100mil em mulheres afro-americanas (HART et al., 1983). No Brasil, os dados sobre prevalência e incidência ainda são escassos, pois até o presente momento, apenas um estudo avaliou a incidência do LES no país. Este estudo, realizado na cidade de Natal (Rio Grande do Norte), demonstrou uma incidência de 8.7/100mil/ano casos novos de LES no ano

de 2000 (VILAR; SATO, 2002).

1.3 ETIOLOGIA E FISIOPATOLOGIA DO LÚPUS ERITEMATOSO SISTÊMICO

Embora a etiologia da doença ainda seja incerta, fatores ambientais, hormonais, imunológicos e genéticos estão associados ao seu desenvolvimento (PISETSKY, 1997; MOK; LAU, 2003). Os mecanismos que geram a doença envolvem uma rede complexa de elementos celulares e moleculares presentes na resposta imune inata e adquirida (SCHUR, 1995; SULLIVAN, 2000; GATEVA et al., 2009; MANOLIO et al., 2009).

A fisiopatologia da doença envolve a produção errônea de autoanticorpos e, também, o depósito de imunocomplexos, como por exemplo, os avaliados na patogênese da glomerulonefrite. Estes mediadores da doença estão envolvidos em danos celulares e teciduais que levam a um processo inflamatório crônico. Sabe-se que a inflamação é um fator posterior à implantação da doença (CRISPÍN et al., 2010).

O antígeno leucocitário humano (HLA) é um fator genético importante envolvido na etiologia do LES. A associação de HLA-DR2 e DR3 com LES é um achado comum em pacientes de diferentes etnias, que então apresentam um risco relativo de duas a cinco vezes de desenvolver a doença (PISETSKY, 1997). Os genes HLA de classe II também têm sido associados com a presença de determinados autoanticorpos, como anti-Smith, anti-SS-A (anti-Ro), anti-SS-B (anti-La), anti-RNP e anti-DNA, corroborando a hipótese que falhas nesses genes contribuam para o aparecimento da doença (SCHUR, 1995).

Além disso, o LES é associado a deficiências herdadas em componentes do sistema complemento como o C1q, C1r/s e C2. A diminuição da atividade do complemento poderia promover maior suscetibilidade à doença ao alterar a neutralização e limpeza de imunocomplexos formados por autoantígenos e autoanticorpos. Quando a carga de antígeno ultrapassa a capacidade de depuração do sistema imunológico, o sistema de autoimunidade poderá ser ativado (ATKINSON, 1986).

A presença de autoantígenos parece ser o elemento deflagrador para o desencadeamento da doença. Processos lesivos em tecidos afetados por uma variedade de causas parecem ser os responsáveis pela exposição destes antígenos ao sistema imune de indivíduos geneticamente suscetíveis, fazendo com que as moléculas que normalmente são encontradas no interior das células do tecido, sejam expostas, induzindo assim, células apresentadoras de antígeno, como linfócito B e fagócitos, a processarem esses autoantígenos e apresentarem a linfócitos T autorreativos (KLINMAN et al., 1991).

Além do reconhecimento inicial, a estimulação da interação de células B e T é facilitada por várias citocinas, como interleucina-10 (IL-10) (KLINMAN et al., 1991). Sabe-se que as células B autorreativas são estimuladas mais facilmente por citocinas, o que contribui para sua expansão clonal no organismo das pessoas que possuem LES (LIOSSIS et al., 1996; LINKER-ISRAELI et al., 1991; MONNEAUX; MULLER, 2002).

Theofilopoulos et al. (2001) mostram um paradigma entre a resposta Th1 e Th2 em que ambas podem estar aumentadas no LES. A resposta imune celular em pacientes com LES, dada pelas células T auxiliares ($TCD4^+$) ou T helper (Th), depende se a doença está em atividade ou não. O padrão Th1 está mais associado à atividade da doença, enquanto que o padrão Th2 se associa ao aumento de produção de autoanticorpos e lesão renal (THEOFILOPOULOS et al., 2001).

O padrão Th1 está associado à inflamação e dano tecidual (resposta mediada por células, principalmente por IFN-γ e IL-12), enquanto o padrão Th2 está associado à atividade da doença e produção de anticorpos (resposta humoral, principalmente mediada por IL-6 e IL-4). Os mecanismos prováveis para isso são a regulação negativa por excesso de citocinas Th2, a interação deficiente entre células apresentadoras de antígenos e células T, os efeitos supressivos das células T citotóxicas ($CD8^+$) e *natural killer* (NK), presença de inibidores de interleucina-2 (IL-2) e regulação diminuída de receptores de IL-2 (GARCIA-COZAR et al., 1996; LAUWERYS; HOUSSIAU, 1998).

Por sua vez, as células Th0 quando estimuladas por IL-6, IL-21 e TGF-beta produzem interleucina 17 (IL-17) e são diferenciadas em Th17, um tipo de padrão associado ao Th1. Esta citocina, quando em níveis elevados, está relacionada à atividade da doença nos pacientes LES (KORN et al., 2009). A IL-17 possui a função de aumentar a resposta inflamatória, por induzir o aumento de produção de interleucina 6 (IL-6) (LINKER-ISRAELI et al., 1991) e interleucina 21 (IL-21) (WONG et al., 2010), e aumentar a sobrevida e proliferação de linfócitos B; além disso, induz a diferenciação de linfócito B em plasmócitos (DOREAU et al., 2009). IL-6 e IL-21 são produzidas por um tipo de célula Th, denominada célula Th folicular (ThF), que é encontrada em regiões germinativas dos linfonodos, cuja ação é sinalizar para as células B produzirem imunoglobulinas (Igs), mudar o isotipo e gerar hipermutação somática. Verificou-se que em modelos murinos a IL-21 e as célula ThF são necessárias para o desenvolvimento da doença (ODEGARD et al., 2008; BUBIER et al., 2009) e que o tratamento com receptor de IL-21 reduz a progressão da doença (HERBER et al., 2007). Este fato demonstra que a população de células B, por meio da influência de citocinas estimulatórias, poderia ser ativada mais facilmente, promovendo maior atividade da

doença.

Os hormônios sexuais também estão associados ao desenvolvimento da doença, porém os mecanismos ainda não estão bem elucidados. A maior prevalência de LES no sexo feminino, a associação com período fértil, e a variação da doença durante o ciclo menstrual e gestação demonstram que influências hormonais estão cada vez mais associadas ao LES (WHITACRE; BLANKENHORN, 1999; ROBERTS et al., 2000).

1.4 DIAGNÓSTICO CLÍNICO E LABORATORIAL DO LÚPUS ERITEMATOSO SISTÊMICO

O diagnóstico da doença é estabelecido quando o paciente apresenta pelo menos quatro dos onze critérios estabelecidos pelo Colégio Americano de Reumatologia (CAR): *rash* malar (na forma clássica de "asa de borboleta"), *rash* discóide (placas avermelhadas na pele com descamação superficial), fotosensibilidade, úlceras orais, serosites, artrites, desordens renais, neuropisiquiátricas, hematológicas e imunológicas, além de anticorpos antinucleares. Estes critérios foram estabelecidos com o objetivo de uniformizar os estudos científicos da doença (HOCHBERG et al., 1997).

Os critérios laboratoriais comumente avaliados para o diagnóstico e monitoramento da doença são: pesquisa de ANAs, pesquisa de anticorpos anti-dsDNA e contra os Antígenos extraíveis do núcleo (ENAs) (DELLAVANCE et al., 2003), dosagem dos componentes do sistema complemento C3 e C4, assim como análises hematológicas, como a contagem de hemácias, leucócitos totais e plaquetas (BORBA et al., 2009).

A pesquisa de autoanticorpos auxilia no diagnóstico do LES, sendo realizado pela metodologia de imunofluorescência indireta (IFI) que utiliza células tumorais epiteliais de faringe (células Hep-2) fixadas em lâmina. A utilização destas células tornou o teste de IFI de alta sensibilidade, sendo indicado para triagem de doenças reumáticas. No entanto, a especificidade do teste é bem menor, o que leva a necessidade de altos títulos de um determinado padrão de anticorpos para garantir o diagnóstico do LES. O padrão mais comum da imunofluoresecência para o LES é o homogêneo, cujos anticorpos do paciente podem apresentar reatividade para o DNA, cromatina e proteínas histonas. Caso estejam presentes outros padrões, como por exemplo, o nuclear pontilhado, torna-se necessária a pesquisa de ENAs, para determinar qual antígeno provocou a produção de autoanticorpos (KAVANAUGH et al., 2000).

A pesquisa de anticorpos contra DNA de dupla fita (anti-dsDNA) é considerada mais específica, porém menos sensível, e é um teste laboratorial utilizado no

diagnóstico e monitoramento da doença. Entretanto, a ausência de anti-dsDNA no sangue não exclui o diagnóstico, pois cerca de 50% dos pacientes com LES não apresentam anti-dsDNA reagente. Além disso, a presença de anti-dsDNA também esta associada à doença renal, sendo considerado um marcador de dano renal (JOSEPH et al,, 2010; KAVANAUGH et al., 2000; HOCHBERG, 1997; KURIEN; SCOFIELD, 2006), podendo ser determinado pela metodologia de IFI ou por *Enzyme-Linked Immunosorbent Assay* (ELISA). Já os ENAs costumam ser determinados pelo teste de ELISA (FERREIRA; ÁVILA, 1996).

Sabe-se que indivíduos com LES podem apresentar comprometimento renal e, dessa forma, deve-se solicitar, concomitantemente à pesquisa de anti-dsDNA, a dosagem de creatinina sérica e urinária, exame de urina I e proteinúria de 24 horas (RAVEL, 1997). Além disso, a avaliação do perfil inflamatório é realizada usualmente mediante a dosagem de proteína C reativa (PCR), alfa-glicoproteína ácida e pela determinação da velocidade de hemossedimentação (HOCHBERG, 1997).

1.5 AVALIAÇÃO DA ATIVIDADE DA DOENÇA

A atividade da doença pode ser determinada de várias maneiras em pacientes com LES. O escore SLEDAI (*Systemic Lupus Erythematosus Disease Activity Index)* é um índice global que inclui 24 critérios clínicos e laboratoriais específicos. A atividade da doença pode variar de 0 a 105 pontos, onde 0 é obtido em casos de ausência de atividade e 105 nos casos com alta atividade da doença (BOMBARDIER et al., 1992; GADMAN et al., 2002). Este índice é utilizado pelo Ambulatório de Reumatologia do Hospital Universitário de Londrina que avalia o comprometimento orgânico e dados laboratoriais nos pacientes com LES (anexo 1).

A determinação da atividade da doença pode ser avaliada também, laboratorialmente, pela dosagem de componentes do sistema complemento, mais comumente de C3 e C4. Os níveis séricos de complemento C3 e C4 podem estar diminuídos nos pacientes com LES em atividade da doença devido ao consumo destes componentes pelos imunocomplexos formados, que podem ativar a via clássica. Observa-se que nos casos de melhora clínica dos pacientes os níveis séricos de complemento aumentam, mas nem sempre voltam aos valores considerados de referência (JOSEPH et al., 2010; KAVANAUGH et al., 2000; HOCHBERG, 1997).

Um outro teste laboratorial solicitado para a determinação da atividade da doença é a pesquisa de anticorpos anti-dsDNA. Comumente este teste é realizado por IFI

sendo o resultado liberado em título. A positividade deste teste e os títulos elevados são indicativos de doença ativa. A remissão da doença, seja pelo tratamento ou não, está associada à diminuição dos títulos de anti-dsDNA e negativação do teste (FERREIRA; ÁVILA, 1996).

1.6 Estresse Oxidativo, Metabolismo de Ferro e Resistência À Insulina no Lúpus Eritematoso Sistêmico

O estresse oxidativo (EO) ocorre quando existe um desequilíbrio entre agentes antioxidantes e oxidantes, sendo ocasionado por radical livre. Este radical é qualquer átomo, molécula ou fragmento de molécula contendo um ou mais elétrons desemparelhados nas suas camadas de valência (HALLIWELL; GUTTERIDGE, 1999). Alguns exemplos de radicais livres são: oxigênio molecular (O_2), radical hidroxil (OH^-), ânion superóxido (O_2^-), radical peroxil (ROO^-), radical alcoxil (RO^-) e óxido nítrico (NO) (SJODIN et al., 1990; ARUOMA, 1994; PEREIRA, 1994; YU, 1994). Esses radicais são encontrados no organismo humano e sua produção está relacionada a processos fisiológicos como a respiração, assim como a processos patológicos como na inflamação, resultando em lesões biológicas (HALLIWELL; GUTTERIDGE, 1999). Os termos substâncias reativas do oxigênio (ROS) e substâncias reativas do nitrogênio (RNS) tem sido usados para descrever compostos com ação de radicais livres. Para se proteger destes processos oxidativos, o organismo dispõe de mecanismos químicos (ácido úrico), bioquímicos (glutationa reduzida-GSH) e biológicos (enzimas: superóxido dismutase-SOD, catalase e glutationa peroxidase-GPX) (WHITE, 1993; YU, 1994).

O processo inflamatório envolvido na fisiopatologia do LES parece levar ao aumento do EO e diminuição da capacidade antioxidante, contribuindo para elevar os danos oxidativos teciduais nestes pacientes. No entanto, este fenômeno precisa ser mais bem elucidado neste modelo de doença, pois a influência de fatores ambientais, medicamentos e estilo de vida podem modificar esse conceito. Além disso, as lesões endoteliais que certamente ocorrem nos pacientes com LES, contribuem para formação de "*foamy cells*" (macrófagos espumosos), pois a peroxidação lipídica, devido a ação dos ROS nas lipoproteínas de baixa densidade (LDL-colesterol) e triacilgliceróis, o que favorece a adsorção de macrófagos ativados em região inflamatória vascular. Assim, há formação de placas de ateroma, contribuindo para o desenvolvimento de doenças cardiovasculares (LINDAHL et al., 2000).

Embora a causa do LES seja multifatorial, tem sido sugerido que o aumento

da produção de ROS e RNS pode favorecer o seu desenvolvimento. O EO pode contribuir para a disfunção das células imunes, produção de autoantígeno e reatividade de autoanticorpos no LES (OATES, 2010). Na doença autoimune, proteínas modificadas oxidativamente são responsáveis por perturbações adicionais, pois representam alvos potenciais para o sistema imunológico, rompendo a tolerância das células B. A função do tecido conjuntivo também pode ser alterada por meio da modificação da estrutura do ácido hialurônico sob a ação de ROS (SHEIKH et al., 2007), tornando-o um alvo para o sistema imune.

No entanto, os efeitos deletérios dos ROS são mais pronunciados em biomembranas e organelas celulares ocasionados pela lipoperoxidação. Como as reações oxidativas são normalmente instáveis, a identificação deste fenômeno se dá pela análise de produtos finais da ação oxidante. O malondialdeído (MDA), um produto final da lipoperoxidação que pode ser quantificado, é um exemplo disso. Este composto pode ser covalentemente ligado às proteínas gerando adutos intra e intermoleculares. Essas alterações podem gerar neoepítopos de proteínas que passam agora a ser reconhecidas pelo sistema imunológico resultando em processo autoimune. As alterações ocorridas podem ocasionar estados de doença aguda e crônica, ser um preditor de risco da doença, e/ou estar envolvidas na progressão da lesão vascular relacionada ao LES (ZHANG et al., 2010). Ben Mansour e colaboradores (2010) demonstraram aumento dos níveis de MDA em pacientes com LES. Outros estudos também demonstraram um aumento nos níveis de MDA avaliados pelo método de determinação das substâncias reativas ao ácido tiobarbitúrico (TBARS), e este foi associado positivamente ao 8-isoprostano, um metabólito da peroxidação lipídica catalizada por radical, cuja estrutura e função se assemelham com a da prostaglandina F2, um importante vaconstritor (MORROW et al., 1996; ABOU-RAYA et al., 2004). O aumento da lipoperoxidação tem sido observado em vários estudos com pacientes com LES com diferentes abordagens metodológicas para a sua avaliação (NUTTAL et al., 2003; KURIE; SCOFIELD, 2003; FROSTEGARD et al., 2005; ABOU-RAYA et al., 2004). No entanto, os resultados ainda são controversos. Tewthanom et al (2008) não demonstraram diferenças na concentração de MDA quando compararam indivíduos controle com pacientes com diferentes graus de gravidade do LES. Os autores sugeriram que um provável motivo para estes dados conflitantes tenha sido o efeito da prednisona na peroxidação lipídica (KUONO et al., 1994).

Estudos demonstram que no LES há peroxidação lipídica associada à formação da placa de ateroma, sendo a oxidação do LDL-colesterol associada a esta formação (MANZI, 2000; LINDAHL et al., 2000; NUTTALL et al., 2003).

A avaliação do estado antioxidante em pacientes com LES tem apresentado resultados conflitantes. Estudos têm demonstrado aumento (TURGAY et al., 2007) ou diminuição (BAE; KIM; SUNG, 2002; TAYSI et al., 2002) da capacidade antioxidante total, enquanto outros não verificaram nenhuma diferença significativa em relação aos indivíduos controles (NUTTALL et al., 2003, ZHANG et al., 2005). No entanto, as metodologias realizadas, o número de pacientes e a atividade da doença foram diferentes nesses trabalhos.

A homeostase de ferritina e ferro têm sido implicados na patogênese de doenças relacionadas à autoimunidade e também em patologias relacionadas à resistência à insulina (RI), como a síndrome metabólica (CHOI et al., 2005; GONZÁLEZ et al., 2006; ZANDMAN-GODDARD G; SHOENFELD, 2007). Sabe-se que a RI mediada no fígado é uma consequência precoce de danos hepáticos ferro-dependentes (ANDREWS, 1999; FERRANNINI, 2000; KIM et al., 2011). A principal função da ferritina é armazenar ferro intracelular de modo compacto, seguro e torná-lo disponível quando necessário. Esta função é devido a uma capacidade muito elevada da proteína de ligação com o ferro e da atividade da enzima ferroxidase, que converte Fe^{++} para Fe^{+++} (AROSIO; INGRASSIA; CAVADINI, 2009). Há evidências de que a ferritina poderia exercer um papel protetor contra os danos mediados pelos radicais livres advindos do oxigênio (AROSIO; INGRASSIA; CAVADINI, 2009). A ferritina é uma proteína que contém e mantém em solução vários milhares de átomos de ferro, que em outra circunstância formariam um precipitado tóxico. O ambiente de maior concentração desta proteína é intracelular, sendo o metabolismo do ferro importante para servir de componente para reações que ocorrem dentro da célula. Esse íon entra e sai da proteína conforme as necessidades celulares (HARRISON; AROSIO, 1996). Porém o ferro e a ferritina são elementos associados ao estresse oxidativo (EO).

O principal papel da ferritina no EO foi evidenciado pela sua participação na reação de Fenton (ZHAO et al., 2001), disponibilizando maior quantidade ferro, e, por conseqüência, aumentando o EO.

Outras funções da ferritina associadas ao EO estão sendo evidenciadas por influenciar nos processos que desequilibram o sistema oxidante-antioxidante, como, por exemplo, um membro do grupo de proteínas que respondem ao estresse e a inflamação. Citocinas inflamatórias, particularmente o TNF-α e a IL-2, regulam positivamente a síntese de ferritina em várias células de mamíferos, incluindo células mesenquimais, hepatócitos e macrófagos-monócitos (MILLER et al. 1991). O elemento regulatório desta resposta, que é específico para a cadeia H-Ferritina e é chamado de FER2, foi identificado com 4.8 Kpb e inicia a transcrição do gene da H-Ferritina que está ligado ao sitio do fator de transcrição

nuclear κB (NFκB) (KWAK et al. 1995). Estes eventos podem ser acompanhados por efeitos indiretos via indução do NO que estimula a diferenciação e ativação da síntese de H e L-Ferritina em nível transcricional (MARZIALI, et al., 1997). Estes eventos parecem gerar um predomínio na indução da H-Ferritina com consequente aumento do EO. Além disso, a observação de que o O^- pode mobilizar o ferro da ferritina levou à sugestão de que a exposição aos radicais livres de oxigênio podem realmente aumentar o *pool* de ferro reativo (reduzido) e exacerbar a lesão oxidante (AROSIO; LEVI, 2002). Além disso, o ferro catalisa a formação de radicais hidroxilas, que são pró-oxidantes poderosos e que atacam lipídios da membrana celular, proteínas e ácidos nucléicos (AROSIO; LEVI, 2002). Portanto, os níveis de ferritina elevados estão associados com o processo inflamatório crônico e podem estar envolvidos no desequilíbrio redox verificado nestas condições. Entretanto, ainda persistem controvérsias sobre o papel funcional da ferritina nestas condições, se pró ou antioxidante.

O musculo esquelético, fígado e pâncreas são os principais responsáveis pelo controle da glicemia. O excesso de glicose, como em um evento pós-prandial, induz a liberação pancreática de insulina que aumenta os transportadores de glicose GLUT-4 no músculo esquelético, e também de GLUT-2 no fígado e com isso há diminuição da glicemia e aumento do consumo e armazenamento de glicose nestes tecidos. Já em situação de diminuição da glicemia, como no jejum prolongado, há liberação pancreática de glucagon que gera glicogenólise no fígado e manutenção nos níveis séricos de glicose. Estes eventos de consumo, armazenamento e liberação de glicose são controlados por hormônios e enzimas intracelulares. Estes processos garantem a manutenção dos níveis séricos de insulina, glucagon e glicose no organismo, e o desequilíbrio entre estes elementos gera a RI (NEWGARD, 2004).

Algumas situações contribuem para o surgimento da RI, como, por exemplo, falhas na sensibilização do músculo esquelético e fígado (SHULMAN et al., 1990), o excesso de peso com aumento de ácidos graxos livres (KIM et al., 2001), o processo inflamatório (BARNES; KARIN, 1997; MILES et al., 1997; SUMMERS et al., 1998; YAMAUCHI et al., 2002) e o EO (KIM et al., 2011).

A RI contribui para a patogênese da síndrome metabólica (SM) por meio da hiperglicemia, hiperinsulinemia compensatória e falha na sensibilização da insulina. Dentre estes, a hiperinsulinemia parece ser o fator mais importante (SIDIROPOULOS; KARVOUNARIS; BOUMPAS, 2008). Estudos mostram elevada prevalência de SM em pacientes com LES no Brasil (VILAR et al., 2006; TELES et al., 2010) e no Mundo (PARKER/ BRUCE, 2010). Chung et al (2007) demonstraram uma prevalência de 44,1% de

RI em pacientes com LES.

Em suma, é possível que a doença inflamatória crônica e o EO inerentes ao LES possam estar diretamente associados ao desenvolvimento de RI nestes pacientes. No entanto, até o presente momento, não há estudos que tenham avaliado se a presença de RI em pacientes com LES aumenta o estresse oxidativo, o que ajudaria na compreensão dos fatores relacionados à manutenção e progressão da doença.

1.7 ÓXIDO NÍTRICO (NO) E LÚPUS ERITEMATOSO SISTÊMICO

O NO é produzido nas células endoteliais pela expressão da enzima óxido nítrico sintase endotelial (eNOS) e é responsável pela vasodilatação e manutenção da função endotelial. A produção de NO também pode ser estimulada pelo aumento na expressão da enzima óxido nítrico sintase induzível (iNOS). Em circunstâncias estimulatórias, como estresse oxidativo, dano endotelial ou tecidual e citocinas pró-inflamatórios, ocorre a produção de NO estimulada por um aumento na expressão da iNOS que é capaz de produzir até 1.000 vezes mais NO do que eNOS (NATHAN, 1997). Portanto, NO produzido por iNOS media a resposta inflamatória, e foi mostrado que causa disfunção vascular em uma série de modelos experimentais (GUNNETT et al., 2003).

Sabe-se que o NO regula as funções das células T em condições fisiológicas, mas a superprodução de NO pode contribuir para a disfunção de linfócitos T, e há estudos relatando aumento da síntese de NO endógeno no LES (NAGY et al., 2010).

A determinação da concentração de metabólitos do óxido nítrico (NOx) em pacientes com LES tem mostrado resultados contraditórios, sendo que alguns autores relataram aumento da produção (BELMONTE et al., 1997; WANCHU et al., 2001), enquanto outros não encontraram qualquer alteração (WIGAND et al., 1997; GONZALEZ-CRESPO et al., 1998).

Oates e colaboradores (2008), demonstraram um aumento dos níveis de NOx em pacientes LES quando comparados com controles, e verificaram, que a atividade da doença estava associado a este aumento. Em outro estudo, Asl e colaboradores (2008) verificaram uma correlação direta entre a presença dos fatores de risco da SM e os valores elevados de NOx no soro de pacientes com LES. Estes dados reforçam a hipótese de envolvimento do NO na disfunção endotelial nestes pacientes.

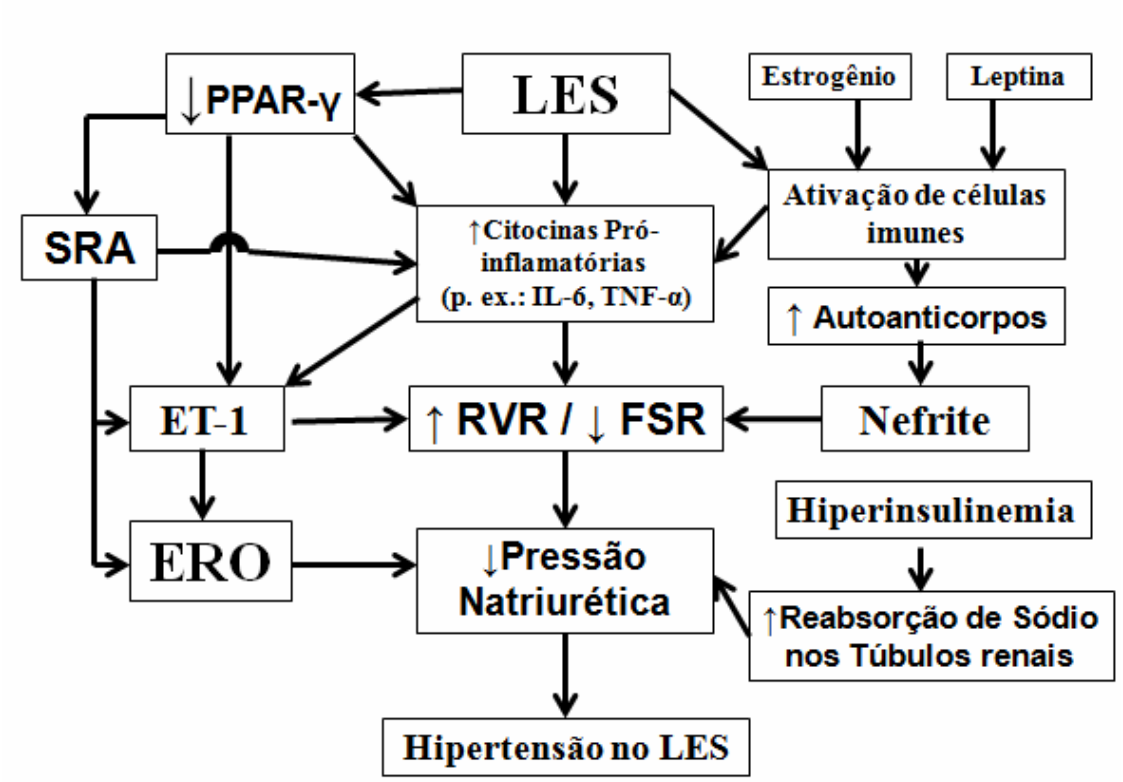
1.8 HIPERTENSÃO ARTERIAL, DOENÇA CARDIOVASCULAR, SÍNDROME METABÓLICA E LÚPUS ERITEMATOSO SISTÊMICO

A hipertensão é considerada um fator de risco maior para progressão de doenças renal, vascular e cardíaca. Numerosos estudos evidenciam a alta prevalência de hipertensão em mulheres com LES, chegando a 70% (BUDMAN; STEINBERG, 1976; PETRI, 2000; AL-HERZ et al., 2003; RAMOS-CASALS et al., 2007). Em contraste, a prevalência de hipertensão em mulheres jovens que não têm LES é de 2,7% entre 20 e 34 anos de idade e 14% entre 35 e 44 anos de idade (ANONYMOUS, 2007). Apesar da forte evidência para a alta prevalência de hipertensão arterial em indivíduos com LES, estudos sobre os mecanismos envolvidos no processo da hipertensão no LES ainda são escassos.

O mecanismo que explica a hipertensão, em grande parte, é a disfunção endotelial levando a resistência vascular periférica, alteração no volume sanguíneo e variações na frequência cardíaca. No LES, há também relatos de um possível papel do sistema imunitário e da inflamação crônica no desenvolvimento de hipertensão (BLAKE et al., 2003; BOOS; LIP, 2006), sendo provável que as citocinas inflamatórias desempenhem um papel central na sua patogênese. Além disso, semelhante a todas as formas de hipertensão estudadas, a função renal alterada desempenha papel importante e é negativamente afetada pela inflamação crônica (RYAN, 2009).

Assim, vários processos patológicos associados ao LES podem influenciar a patogênese da hipertensão, como as alterações renais (RYAN; McLEMORE Jr; HENDRIX, 2006), a disfunção endotelial (WILLIAMS et al., 2002), as alterações endócrinas e metabólicas (DZAU; RE, 1994), o EO (LASSEGUE; GRIENDLING, 2004; MANNING; TIAN; MENG, 2005) e as citocinas pró-inflamatórias (RUDDY et al., 2004; SESSO et al., 2007; HARRISON et al., 2010). Portanto, há várias vias possíveis para o desenvolvimento da hipertensão no LES, sendo que as pesquisas desenvolvidas até o momento tem investigado principalmente as alterações na resistência periférica e volume sanguíneo (disfunção endontelial), e também os envolvimentos inflamatórios e renais (Fig.1).

Figura 1 - Diagrama das possíveis vias envolvidas na hipertensão em pacientes com LES. SRA, sistema renina-angiotensina; RVR, resistência vascular renal; FSR, fluxo sanguíneo renal;



PPAR- γ, receptor proliferador ativado de peroxissoma-γ; ERO, espécies reativas de oxigênio; ET-1, endotelina-1.
Fonte: Adaptado de Ryan e Yanai (YANAI et al., 2008; RYAN, 2009).

Os pacientes com LES dispõem hoje de considerável arsenal de medicamentos para tratamento de sua doença, incluindo antiinflamatórios não esteróides, corticosteróides e outros imunosupressores, antimaláricos, imunoglobulina endovenosa e anticorpos anti-CD20, podendo ainda ser realizado, nos casos aplicáveis, o transplante de medula óssea. Como conseqüência direta do aumento do conhecimento e das possibilidades de tratamento de pacientes com LES, a sobrevida desses pacientes tem aumentado consideravelmente nos últimos 40 anos, levando ao aparecimento e reconhecimento de novas causas de morbidade e mortalidade, entre elas a doença aterosclerótica (TELLES et al., 2007).

Alguns estudos demonstram um aumento da morbidade e mortalidade no LES e a causa pode ou não estar relacionada com a própria doença. Em um estudo realizado na Europa, Cerveza e colaboradores (2003) avaliaram a mortalidade dos pacientes com LES durante 10 anos e constataram que durante os primeiros 5 anos houve aumento da mortalidade ocasionada, principalmente, pela atividade da doença e infecções (29% cada), enquanto que após 5 anos, as mortes estavam relacionadas à distúrbios cardiovasculares (26%).

Petri (2000) relata que, embora a mortalidade aguda esteja declinando, há muitos pacientes que morrem prematuramente e a causa de morte em pacientes com tempo de duração da doença superior a 5 anos é a doença arterial coronariana (DAC).

No *Framingham Offspring Study*, mulheres com LES em idade entre 35 a 44 anos apresentaram 52 vezes mais risco de infarto do miocárdio do que mulheres saudáveis de idade similar (MANZI et al., 1997). De modo semelhante, um estudo na Suécia, avaliou 4700 indivíduos e mostrou uma razão de morte por DAC de 15,9 vezes em pacientes com LES (BJORNADAL et al., 2004), sendo as principais causas os danos vasculares e o perfil lipídico alterado (NUTTALL et al., 2003).

A SM é um transtorno complexo representado por um conjunto de fatores de risco cardiovascular, usualmente relacionados à deposição central de gordura e à resistência periférica à ação da insulina (REAVEN, 1988).

Os fatores de risco cardiovascular presentes na SM incluem obesidade central, dislipidemia, pressão arterial elevada e distúrbio no metabolismo de glicose. A SM é um preditor independente de morbidade e mortalidade cardiovascular (FORD et al., 2002; REILLY; RADER, 2003; KIP et al., 2004). Reaven, em 1988, denominou a doença com o termo "síndrome X" para descrever a associação entre a RI, diabetes mellitus tipo 2, hipertensão arterial e doenças cardiovasculares e, em 1998, a primeira definição formal da SM foi proposta pela Organização Mundial da Saúde (OMS) (ALBERTI et al., 1998).

Outra definição para classificação de SM foi proposta pelo "*Executive Summary of the Third Report of the National Cholesterol Education Program"* (NCEP) *"Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults*" (ATPIII) em 2001 (JACOBS JR, 2001). De acordo com esta definição, o diagnóstico de SM seria estabelecido quando pelo menos 3 dos 5 critérios descritos no quadro 1 estiverem presentes.

Quadro 1 - Definição de Síndrome Metabólica segundo *Adult Treatment Panel III* (ATP III)

Devem estar presentes pelo menos 3 dos 5 components abaixo descritos:
Glicose de jejum ≥ 100 mg/dL
Circunferência abdominal (CA)
Homens > 102 cm
Mulheres > 88 cm
Triacilglicerol ≥ 150 mg/dL
HDL colesterol
Homens < 40 mg/dL
Mulheres < 50 mg/dL
Pressão arterial ≥ 130/85 mm Hg (ou uso de anti-hipertensivos)

Fonte: Adaptado de Reaven, 2006.

Estudos em diferentes populações, como a mexicana, a norte-americana e a asiática, revelam prevalências elevadas de SM entre 12,4% a 28,5% em homens e 10,7% a 40,5% em mulheres, variando de acordo com o critério utilizado e as características da população estudada (GILES, 2003; AGUILAR-SALINAS et al., 2004; FORD; OH et al., 2004; HU et al., 2004).

Na população brasileira, alguns estudos têm descrito uma prevalência de SM entre 10 a 40%, sendo maior entre as mulheres, em torno de 30%, do que em homens, em torno de 20% (BARBOSA et al., 2006; SOUZA et al., 2003; OLIVEIRA et al., 2006; VELASQUEZ-MELENDEZ et al., 2007).

Em um estudo de revisão, os autores mostraram alta prevalência de SM em pacientes com LES em países desenvolvidos, variando de 18 a 38,2% (PARKER; BRUCE, 2010). No Brasil, embora os estudos ainda sejam escassos, os dados parecem não ser muito diferentes e relatam prevalência em torno de 20 a 32% (VILAR et al., 2006; TELLES et al., 2010). Nosso grupo, em um estudo anterior, demonstrou uma freqüência de 41% de SM, de acordo com os critérios do ATP III, em pacientes com LES atendidos no Hospital Universitário (HU) de Londrina, Paraná, Brasil (LOZOVOY et al., 2011a). A inflamação parece ter um papel importante no desenvolvimento da SM e tem sido demonstrada uma associação direta entre o processo inflamatório e a RI (FESTA et al., 2000), sendo este um dos possíveis elos para explicar a associação entre LES e SM. Além disso, o tratamento com corticosteróides pode também favorecer o desenvolvimento de SM em pacientes com LES (KARP et al., 2008).

Parker e Bruce (2010) descreveram aumento do risco cardiovascular em

pacientes com LES quando estes apresentavam concomitantemente SM. Vários outros autores corroboraram esse dado (VILAR et al., 2006; CHUNG et al., 2007; VADACCA et al., 2009), enquanto Chung et al (2009) encontraram uma prevalência de 44,1% de RI em pacientes com LES.

Assim, a RI e a SM são fortes candidatos a justificar o aumento da DAC em pacientes com LES. Além disso, vários estudos têm demonstrado que pacientes com LES apresentam RI mais grave em comparação com a população geral (EL-MAGADMI et al., 2006; SADA et al., 2006; CHUNG et al., 2007).

Além de obesidade e RI, os indivíduos com SM também apresentam importante disfunção endotelial e aceleramento da aterosclerose. Vários fatores têm sido associados ao desenvolvimento da SM, tais como: 1) EO, 2) adiposidade visceral e produção desequilibrada, pelo tecido adiposo, de citocinas pró e antiinflamatórias (adiponectina) resultando em inflamação de baixo grau, 3) a diminuição da biodisponibilidade do NO resultando em disfunção endotelial e 4) hiperuricemia. Sabe-se que a inflamação é uma manifestação do EO e as vias que geram os mediadores inflamatórios, tais como moléculas de adesão e interleucinas, são também induzidas pelo EO. A ativação do processo inflamatório pode resultar, por sua vez, em mais EO (WILLIAMS et al., 2002; CERIELLO; MOTZ, 2004; DANDONA et al., 2005, LOZOVOY et al, 2011 b). Portanto, a RI, o EO e o NO parecem ser fatores comuns para o desenvolvimento da SM no LES.

2 JUSTIFICATIVAS

Assim, considerando que, até o momento, não é do nosso conhecimento trabalhos na literatura que tenham verificado:

1º) A freqüência de RI em pacientes com LES na população brasileira;
2º) A diferença de freqüência de RI em pacientes com LES em atividade ou não;
3º) A freqüência de hipertensão arterial em pacientes com LES em atividade ou não;
4º) A avaliação concomitante dos possíveis fatores que interferem no aumento da pressão arterial em pacientes com LES em atividade ou não.
5º) A associação entre o EO e o metabolismo de ferro em pacientes com RI e LES.

O presente trabalho tem como objetivos:

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar a freqüência de resistência à insulina (RI) e hipertensão arterial em pacientes com LES em atividade ou não, sem comprometimento renal, e verificar os possíveis fatores envolvidos.

3.2 OBJETIVOS ESPECÍFICOS

a) Determinar a frequência de RI em indivíduos controles saudáveis e em pacientes com LES
b) Verificar se a atividade da doença aumenta a frequência e a razão de chance (*Odds Ratio*) de pacientes com LES apresentarem RI e síndrome metabólica.
c) Avaliar os marcadores lipídicos e protêicos de EO, os níveis séricos de NOx e o estado antioxidante em indivíduos controles saudáveis e em pacientes com LES.
d) Verificar o envolvimento da resposta imune Th1/Th2/Th17 na hipertensão arterial em indivíduos controles saudáveis e em pacientes com LES em atividade ou não.
e) Avaliar o metabolismo de ferro em indivíduos controles saudáveis e em pacientes com LES
f) Verificar se a presença de RI aumenta o EO em pacientes com LES.
g) Verificar se há associação entre as alterações no metabolismo de ferro, o EO e a RI em pacientes com LES.

4 TRABALHOS DESENVOLVIDOS

Foram desenvolvidos 3 artigos científicos para apresentar e discutir os resultados obtidos. Esses artigos foram formatados de acordo com as instruções para autores determinadas pelos periódicos científicos "LUPUS" (artigo 1), "Journal of Translational Medicine" (artigo 2) e "Journal of Scandinavian Rheumatology" (artigo 3). Essas normas estão no anexo 4.

Artigo 1: **INCREASED INSULIN RESISTANCE AND METABOLIC SYNDROME FREQUENCY IN BRAZILIAN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS: COMPARISON BETWEEN ACTIVE AND INACTIVE DISEASE**

Artigo 2: **INFLUENCE OF TH_1/TH_2/TH17 CYTOKINES LINEAGE, NITRIC OXIDE, OXIDATIVE STRESS, AND INSULIN RESISTANCE ON BLOOD PRESSURE IN ACTIVE AND INACTIVE SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS WITHOUT NEPHRITIS**

Artigo 3: **RELATIONSHIP BETWEEN IRON METABOLISM, OXIDATIVE STRESS AND INSULIN RESISTANCE IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS**

4.1 ARTIGO 1: INCREASED INSULIN RESISTANCE AND METABOLIC SYNDROME FREQUENCY IN BRAZILIAN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS: COMPARISON BETWEEN ACTIVE AND INACTIVE DISEASE

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Insulin resistance and metabolic syndrome in lupus

ABSTRACT

Patients with systemic lupus erythematosus (SLE) have higher insulin resistance and metabolic syndrome prevalence than the general population. However, to date, insulin resistance and metabolic syndrome prevalence in active and inactive disease have not been reported. The objectives of this study were to verify the prevalence of insulin resistance and metabolic syndrome in Brazilian patients with SLE and to analyze whether disease activity interferes with the aforementioned conditions. The study included 130 controls and 74 SLE patients. SLE patients were divided in active (36 patients) and inactive (38 patients) disease. Insulin resistance prevalence measured by HOMA-IR (Homeostasis Model Assessment - Insulin Resistance) was verified in 38 (51.35%) patients with SLE and in 38 (29.23%) controls (p=0.0017, OR: 2.556, IC 95%: 1.413-4.621), whereas MetS prevalence measured by ATP-II (Adult Treatment Panel III) was observed in 25 (33.78%) patients with SLE and in 17 (13.08%) controls (p<0.0001, OR=4.644, CI 95%: 2.644-9.625). Insulin resistance was verified in 23 (63.89%) patients with active SLE and in 15 (39.47%) patients with inactive SLE (OR: 2.781, IC 95%: 1.568-4.932, p=0.0004), whereas 15 (41.67%) patients with active SLE met the criteria for metabolic syndrome compared with 10 (26.32%) with inactive SLE (OR: 2.061, IC 95%: 1.133-3.748, p=0.0169). Body mass index and corticosteroids use significantly increased in the active group. This study reinforces the higher risk for

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developing insulin resistance and metabolic syndrome in patients with SLE, especially in those with active disease, and support the role of insulin resistance and corticosteroid use as the main links between disease activity and metabolic syndrome.

Key Indexing Terms: systemic lupus erythematosus, insulin resistance, metabolic syndrome, glucocorticoids.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by multisystem organ involvement and by high titers of autoantibodies against several nuclear and cytoplasmatic antigens.[1]

Whereas the impact of infections and active disease on mortality has diminished dramatically over the years, due to intensive treatment, cardiovascular disease (CV) has emerged as the leading cause of death in these patients.[2] Increased risk of coronary heart disease (CHD) in SLE is not explained by the classic CHD risk factors.[3-6]

Reaven[7] have proposed the concept of metabolic syndrome (MetS) in 1988. Since then, many researchers believe that insulin resistance is the pathophysiological process underlying the clustering of CV risk factors in MetS and that insulin resistance increases the values of clinical diagnosis of MetS.[8-11] Therefore, insulin resistance and MetS are clearly strong candidates to justify the increase in CHD in patients with SLE.

Several studies have demonstrated that SLE patients have more severe insulin resistance compared with the general population.[6,12-14] Insulin resistance may contribute to the pathogenesis of MetS through hyperglycemia, compensatory hyperinsulinemia, and imbalanced insulin action. Among them, hyperinsulinemia seems to be the most important factor.[15] Chung et al.[13] found a prevalence of 44.1% of insulin resistance in patients with SLE. Several studies have also shown high MetS prevalence in patients with SLE in developed countries. MetS classification in patients with SLE, according to National Cholesterol Educational Program (NCEP ATP-III), showed a prevalence of 16% in Netherlands[16], from 17% to 20% in Spain[17,18], 18% in the United Kingdom[6], and 29.4% and 32.4% in the United States[13], when the authors used NCEP ATP-III or World Health Organization (WHO) classification, respectively. The later classification requires direct determination of insulin resistance.[19] In Italy[20], also using World Health Organization (WHO) classification, it was

found a prevalence of 28%. In Latin American countries, this scenario is not very different, and MetS classification according to American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI) showed a prevalence which ranged from 28.6% in Argentina[21] to 38.2% in Puerto Rico.[22] In Brazil, a South American emerging country with a continental dimension, the classification according to NCEP ATP-III reports have found a prevalence of 20% in Northeast[23], and 32.1% in Southeast.[24]

There are several mechanisms by which higher severity and frequency of insulin resistance in SLE patients could be explained, such as obesity, inflammatory markers, and the medication to treat SLE patients, especially glucocorticoids. However, studies, which have focused on lupus disease activity, another mechanism that could contribute to insulin resistance and MetS, have been scarce and controversial. We are aware of only one study with SLE pediatric patients in which they were classified in active and inactive disease.[25] On the other hand, association between MetS and disease activity has been reported in some reports[16,18,24], but not in others.[6,13]

Although some studies have verified a correlation between insulin resistance and metabolic syndrome with disease activity, little is known about disease activity participation in those two conditions. Therefore, the objectives of the present study were to verify the prevalence of insulin resistance and MetS in patients with SLE and to analyze whether disease activity is associated with the aforementioned conditions.

SUBJECTS AND METHODS

Subjects

The study included 204 subjects. One hundred and thirty healthy individuals were selected among blood donors of the Blood Bank of the University Hospital of Londrina and 74 patients with SLE were selected among the ambulatory of Rheumatology of the University Hospital of Londrina, Paraná, Brazil, to participate in the study. They were paired by sex, age, ethnicity, and body mass index (BMI). Systemic Lupus Erythematosus was diagnosed using the American College of Rheumatology (ACR) 1997 revised criteria[26]. Patients with SLE were also divided in two groups: with active disease (n=36) or inactive disease (n=38). The following parameters were used to classify SLE as active: SLE Disease Activity Index (SLEDAI) score ≥ 6 [27], and/or decreased C3 (<90mg/dL) and/or decreased C4 complement (<

10 mg/dL), and/or positive anti-dsDNA (titre ≥ 1/10).[28,35] MetS was defined following the Adult Treatment Panel III criteria[29], when three of the following five characteristics were confirmed: 1) Abdominal obesity: waist circumference ≥ 102 cm in men and ≥ 88 cm in women; 2) Hypertriglyceridemia ≥ 150 mg/mL; 3) Low levels of HDL cholesterol: ≤ 40 mg/dL in men and ≤ 50 mg/dL in women; 4) High blood pressure: ≥ 130/85 mmHg; and 5) High fasting glucose: ≥ 110mg/dL or use oh hypertensive drugs.

Information on lifestyle factors and medical history were obtained at clinical evaluation. Disease duration, organ involvement, values of C3 and C4 complement, anti-double-stranded DNA (anti-dsDNA), and use of non-steroidal anti-inflammatory drugs, corticosteroids, antimalarials, oral contraceptives, and antihypertensive medications were recorded for each patient. All patients were receiving prednisone at the time of inclusion, thus predinisone-equivalent calculation was not required. They had been taking the same prednisone dose at least for the past 4 months. No patient with SLE presented proteinuria. Nutritional status of patients was similar to that of the control group. None of the subjects were receiving a specific diet. The individuals of both groups did not drink alcohol regularly. None of the participants in the study presented heart, thyroid, renal, hepatic, gastrointestinal or oncological diseases, and none were receiving estrogen replacement therapy, drugs for hyperlipidemia, hyperglycemia or antioxidant supplements. All patients gave written informed consent, and the study protocol was fully approved by the Ethical Committee of the University of Londrina (Paraná, Brazil).

Anthropometric and Blood Pressure Measurements

Body weight was measured to the nearest 0,1 kg by using an electronic scale, with individuals wearing light clothing, but no shoes, in the morning.; height was measured to the nearest 0,1 cm by using a stadiometer. Body mass index was calculated as weight (kg) divided by height (m) squared. Three blood pressure measurements taken with a minute interval between them after the subject had been seated were recorded. The mean of these measurements was used in the analysis.[30] We considered the current use of antihypertensive medication as an indication of high blood pressure.

Biochemical and Immunological Biomarkers

After fasting for 12 hours, the patients underwent the following laboratory blood analysis: glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, uric

acid, evaluated by a biochemical auto-analyzer (Dimension Dade AR Dade Behring, Deerfield, IL, USA), using Dade Behring® kits; Plasma insulin levels were determined by plasma insulin levels were determined by chemiluminescence microparticule immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA). The homeostasis model assessment insulin resistance (HOMA-IR) was used as a surrogate measurement of insulin sensitivity[31]. HOMA-IR = insulin fasting (μU/ml) X glucose fasting (nmol/L) / 22.5. Insulin resistance was considered when HOMA-IR ≥ 2.114.[32]

Serum complement factors C3 and C4 levels and hsCRP (highly sensitive CRP) were measured using a nephelometric assay (Behring Nephelometer II, Dade Behring, Marburg, Germany). Serum TNF-α, IL-6 and adiponectin levels were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a commercial immunoassay (R&D System). Anti-double-stranded DNA (anti-dsDNA) antibodies were determined by immunoflurescence using *Crithidia lucilliae* kinetoplast assay (Obelis S/A, Brussels, Belgium).

Statistical analysis

Distribution of sex, race, and smoking was analyzed with Fisher's exact test or chi-square test. Comparisons between patients with active or inactive SLE were done using the Mann-Whitney test and data were expressed as the median (25-75 percentiles). Correlations were evaluated by Spearman's rank correlation. It was performed a multivariate regression analysis with a view to determining which variables showed the strongest relationship with activity disease. The results were considered significant when $p<0.05$. A statistical analysis program (Graph Pad Prism version 4.0) was used for evaluations.

RESULTS

Clinical characteristics of the patients with SLE are shown in table 1. The majority of patients did not present clinical signs and symptoms of disease. In relation to the control group, patients with SLE presented significantly higher systolic ($p=0.006$) and diastolic ($p=0.0007$) blood pressure, triacylglycerol ($p=0.039$) levels, insulin ($p<0.0001$) levels, and HOMA-IR ($p=0.0006$), and significantly lower blood glucose levels ($p=0.017$) (Table 2). Patients with SLE and the control group had no differences in WC, and serum total cholesterol, HDL cholesterol, LDL cholesterol, hsCRP, and adiponectin concentrations (Table

2).

Insulin resistance prevalence was verified in 51.35% in patients with SLE and 29.23% in the control group (p=0.0017, OR: 2.556, IC 95%: 1.413-4.621), whereas MetS prevalence was 33.78% in patients with SLE and 13.08% in the control group (p<0.0001, OR=4.644, CI 95%: 2.644-9.625) (Figure 1).

Patients with active or inactive disease were paired by gender, age, and ethnicity. Patients with active disease had higher BMI (p=0.003) and WC (p=0.035) when compared to control subjects. Multivariate analysis showed association between BMI and disease activity (p=0.0184), but not with WC. As expected, patients with active SLE were taking higher prednisone doses (p=0.041), had higher SLEDAI score (p<0.0001), and presented serum C3 (p<0.0001) and C4 (p=0.0002) lower concentrations (Table 3). Patients with active SLE presented significantly higher serum insulin concentration (p=0.048) and HOMA-IR (p=0.030) and a trend to lower blood glucose levels (0.058) compared with those patients with inactive SLE. There were no differences between the groups with regard to systolic and diastolic blood pressure, triacylglycerol, total cholesterol, HDL cholesterol, and LDL cholesterol concentrations (Table 3).

Insulin resistance was verified in 63.89% patients with active SLE and in 39.47% patients with inactive SLE (OR: 2.781, IC 95%: 1.568-4.932, p=0.0004) (Figure 2), whereas 41.67% patients with active SLE met the criteria for metabolic syndrome compared with 26.32% with inactive SLE (OR: 2.061, IC 95%: 1.133-3.748, p=0.0169) (Figure 2).

In relation to the inflammatory markers, patients with active SLE had higher CRP concentration (p=0.030) than patients with inactive SLE (Table 3). There were no differences between the groups in α_1 acid glycoprotein, IL-1, IL-6, TNF-α and adiponectin concentrations. Also, IL-1, IL-6, and TNF-α concentrations were not associated with any disease activity parameter (data not shown). However, adiponectin concentration was directly correlated with daily prednisone doses (r=0.437, p=0.018) in active SLE patients (figure 3).

DISCUSSION

The major findings of the current study are that patients with SLE have an increased risk for developing insulin resistance (2.5 times) and MetS (4.6 times) than a control group. In

addition, even a mild to moderate activity in patients with SLE is associated with an increased risk for developing insulin resistance (2.8 times) and MetS (2.1 times) compared with inactive SLE patients. The data from the present study permit to suggest that disease activity shown by increased SLEDAI score and decreased serum C3 and C4 levels has also an important role in the development of insulin resistance in SLE patients.

Although previous studies that have also demonstrated enhanced levels of fasting insulin[6,12-14], enhanced prevalence of insulin resistance[13], and MetS[19] in SLE patients, to our knowledge the present study showed the highest prevalence of MetS in patients with SLE to date. The higher insulin resistance and MetS prevalence which were found in the present study may be explained by the following reasons: firstly, many studies[17,18,22] have verified that lupus patients was associated with lower income, a finding which was also verified in most of our patients. Secondly, as MetS prevalence is increasing over time in the general population[33], it is likely that it is also increasing in SLE patients.

Of note, SLE patients presented lower blood glucose levels, but higher insulin and HOMA-IR levels in relation to control subjects. The same trend was observed when active SLE patients were compared to inactive SLE patients. It can be assumed that metabolic derangement in these patients with inactive or lupus with mild activity are in an initial phase, as increased insulin production is still sufficient to maintain blood glucose levels within normal range, although insulin resistance shown by HOMA-IR seems to be established. Noteworthy, differently from insulin resistance results which showed higher levels in active disease, all components of NCEP ATP-III classification used in the present study were not different in active or inactive disease despite a higher MetS prevalence, showing the importance of insulin resistance in the development of these components. Our data are in accordance with Reilly et al.[11] who suggested the use of biomarkers of insulin resistance in addition to ATP-III criteria in assessing CV risk.

The present study is in accordance with previous studies, which also have found an association between lupus disease activity and MetS. This association has been shown with SLEDAI score[22,24,34], whereas with serum C3 levels, it has been demonstrated inverse[18] and direct association[16] with MetS. This apparent paradox can be explained because serum C3 and C4 levels may, in some circumstances, act as an acute-phase reactant protein[35]. On the other hand, no association between SLE activity and MetS was found by others authors[6,13]

It has been proposed that obesity[32], corticosteroid therapy[36], and chronic inflammatory

process[37,38] are involved in MetS found in SLE patients. BMI and WC were higher in active disease when compared with inactive disease and multivariate analysis confirmed the association between BMI and disease activity. These data suggest that increased body mass probably due to glicocorticoids use also contributes to increased risk of developing metabolic syndrome in active disease.

The association between glucocorticoids and MetS is controversial. Several reports have shown association between prednisone use above 10 mg/d and MetS[22,33,39] or with intravenous prednisolone[16]. However, other reports have not found any association[6,13,24]. In between, Posadas-Romero et al.[25] found that prednisone explained 15.6% in the variance of insulin levels.

Roman et al.[5] have demonstrated an association between atherosclerosis and less immunosuppressive therapy arguing strongly on the importance of chronic inflammation in SLE patients. Cytokines levels are elevated both in active and inactive periods of SLE, indicating that there is a low-grade chronic inflammation, which can be increased during exacerbation in SLE[38]. Systemic chronic inflammation has been proposed to have a prominent role in the pathogenesis of insulin resistance[37,38]. The proinflammatory state induces insulin resistance, leading to clinical and biochemical manifestations of the MetS. The resistance to insulin action promotes further inflammation through an increase in free fatty acids concentration and interferes with the anti-inflammatory effects of insulin[40]. Inflammatory cytokine TNF-α can induce insulin resistance and suppression of Glut4 expression. IL-6 also inhibits insulin signal transduction in hepatocytes[15]. It was not verified any difference in active and inactive SLE patients in pro inflammatory cytokines. Furthermore, in the current study, none cytokine was correlated with disease activity markers (data not shown). Serum TNF-α and IL-6 concentrations, among others, have been reported to relate to disease activity in SLE, in special in patients with lupus nephritis[41]. In the present study, none patient had lupus nephritis and in active SLE group (median SLEDAI score 4) most patients had mild active disease status. In addition, patients with active disease were taking higher daily prednisone doses than inactive patients, which could favor the similar data observed in relation to the pro inflammatory cytokines between the groups. Nevertheless, CRP showed higher values in active SLE. Other studies have shown association between CRP and MetS[13,18,42]. Besides being considered a predictor of both diabetes mellitus and MetS in the general population, higher CRP levels seem to be associated with both increases in SLE activity and disease damage indexes[19].

Adiponectin is an adipokine that has anti-diabetic, anti-inflammatory and anti-atherogenic effects. Adiponectin acts as an insulin sensitizer and decreases the inflammatory response induced by TNF-α.[43]. Reduction in adiponectin levels has been considered an important pathophysiological mechanism associated to insulin resistance, type 2 diabetes mellitus, and metabolic syndrome[43]. Even in SLE, patients with insulin resistance showed significantly lower adiponectin levels than patients without insulin resistance[12]. In the current study, adiponectin levels were not decreased in SLE patients compared to controls, even though MetS prevalence was significantly higher in the group of patients with SLE. Also, adiponectin concentration did not differ between active and inactive SLE patients. Similarly, to our data, Vadacca et al.[20] have not found differences in adiponectin levels in SLE patients in relation to controls. However, in contrast with this reduction of adiponectin in metabolic conditions associated with excess adipose tissue, some reports have shown an increase in adiponectin concentration in patients with SLE[14,44] as well as in other inflammatory conditions[45]. In the meantime, our data are in agreement with a previous study which has also demonstrated a positive correlation between adiponectin and daily prednisone doses.[46]. Jang et al.[47] suggested that after dexamethasone, increased levels of adiponectin may be a compensatory mechanism in the setting of reduced insulin sensitivity.

When looking at the results of this study, the following limitations have to be considered. First, the adipocytokines were measured only once during the study and multiples measurements of adipocytokines over time are likely to provide additional information. Second, our population included patients with SLE with relatively low disease activity, and therefore, our findings may not be applicable to patients with severe active disease. Nevertheless, the main strength of the present study is its original design, which allowed investigating for the first time, to our knowledge, the increased risk of moderately active SLE patients in developing insulin resistance and MetS when compared with inactive disease patients.

In conclusion, this study reinforces the higher risk for developing insulin resistance and MetS in patients with SLE, especially in those with active disease, and support the role of insulin resistance and corticosteroid use as the main links between disease activity and MetS. Routine inclusion of simple indices of insulin resistance in SLE patients is strongly suggested as well as non pharmacological and pharmacological measures when necessary to improve insulin sensitivity. The development of selective anti-inflammatory steroids, less susceptible to undesirable metabolic effects, is still awaited.

Acknowledgments

This work was supported by the University of Londrina Research Funds (FAEPE).

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Table 1: Clinical and laboratory profile of patients with SLE

	SLE Patients (n=74)	Frequency (%)
Disease Duration (years/SD)*	8.5 (4.0-12.3)	---
SLEDAI Score*	2 (0-4)	---
SLEDAI Score > 6	14	10.4
Anti-dsDNA Positive	15	11.1
C3 (<90mg/dL)	13	9.6
C4 (<10 mg/dL)	5	3.7
Therapy		
Prednisone	71	95.9
Antimalarials	44	54.5
Current Immunosuppressive	22	29.7
ACE inhibitors	9	12.2

Mann-Whitney test. *Data are expressed median (25%-75%). SLE, Systemic Lupus Erythematosus; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; NSAIDs, nonsteroidal anti-inflammatory drugs; ACE, angiotensin-converting enzyme

Table 2: Clinical and laboratory characteristics of patients with systemic lupus erythematosus (SLE) and controls.

	Control (n=130)	SLE (n=74)	P value
Gender (male/female)*	9/121	7/67	0.457
Caucasian/not Caucasian*	106/24	59/15	0.752
Age (years)	39.0 (31.0-46.0)	42.0 (29.0-51.0)	0.415
BMI (kg/m^2)	25.00 (22.91-28.16)	26.84 (22.86-30.86)	0.101
WC (cm)	90.0 (84.5-100.0)	93.0 (84.0-104.5)	0.423
Systolic Blood Pressure (mmHg)	110.0 (101.0-125.0)	119.5 (109.5-130.0)	0.006
Diastolic Blood Pressure (mmHg)	71.0 (65.5-80.0)	78.0 (70.5-85.5)	0.0007
Triacylglicerol (mg/dL)	93.0 (68.0-125.0)	106.0 (78.0-152.0)	0.039
Cholesterol (mg/dL)	196.0 (173.0-218.0)	188.0 (162.0-210.0)	0.127
HDL cholesterol (mg/dL)	55.0 (48.0-66.0)	53.0 (45.0-60.0)	0.197
LDL cholesterol (mg/dL)	116.0 (94.5-137.0)	107.0 (88.5-127.5)	0.094
Glucose (mg/dL)	88.0 (83.0-95.0)	84.0 (80.0-92.5)	0.017
Insulin (μU/mL)	7.20 (5.15-10.40)	9.90 (7.30-13.10)	<0.0001
HOMA-IR	1.60 (1.13-2.32)	2.20 (1.55-2.96)	0.0006
C-reactive protein (mg/dL)	2.07	1.95	0.647

	(0.88-3.88)	(0.80-1.95)	
Adiponectin (mg/dL)	6.47	5.95	0.151
	(4.77-9.17)	(4.21-8.10)	

Mann-Whitney test. *Chi-square test. Data are expressed median (25%-75%). SLE, Systemic Lupus Erythematosus; BMI, Body mass index; WC, Waist Circunference; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; HOMA-IR, Homeostasis Model of Assessment Insulin Resistance.

Table 3: Disease activity parameters, anthropometric measurements, lipid profile, carboidrate metabolism, and insulin resistance and metabolic syndrome prevalence in active and inactive SLE patients

Parameters	Inactive SLE (n = 38)	Active SLE (n = 36)	P value
Gender (male/female)*	4/34	3/33	1.000
Caucasian/not Caucasian**	31/7	28/8	0.684
Disease Duration (years)	7.0 (4.0-10.0)	10.0 (4.75-15.5)	0.201
Age (years)	37.0 (28.5-52.0)	43.0 (29.5-50.5)	0.6188
BMI (kg/m^2)	24.5 (21.8-27.8)	28.8 (26.1-32.9)	0.003
WC (cm)	90.0 (83.0-94.0)	101.5 (84.0-107.0)	0.035
Antimalarials (Yes/No)	20/18	21/15	0.619
Prednisone (mg/day)	5.50 (5.00-15.00)	12.5 (7.50-20.0)	0.041
SLEDAI	0 (0-0)	4 (2-6)	<0.0001
C3 (mg/dL)	121.0 (113.5-140.5)	99.2 (87.7-118.0)	<0.0001
C4 (mg/dl)	24.1 (18.7-28.7)	15.9 (13.4-21.3)	0.0002
Systolic Blood Pressure (mmHg)	120.0 (108.5-129.0)	119.0 (109.5-132.0)	0.741
Diastolic Blood Pressure (mmHg)	78.0 (70.0-85.0)	78.5 (73.0-88.5)	0.305
Triacylglicerol (mg/dL)	102.0 (71.5-144.0)	123.0 (85.5-160.0)	0.114

Cholesterol (mg/dL)	193.5 (168.0-204.5)	182.0 (161.0-216.5)	0.986
HDL cholesterol (mg/dL)	52.5 (45.7-68,8)	52.5 (43.0-59.0)	0.593
LDL cholesterol (mg/dL)	107.0 (91.5-127.5)	110.0 (85.0-133.0)	0.880
Glucose (mg/dL)	86.5 (81.5-94.5)	83.0 (80.0-89.0)	0.058
Insulin (μU/mL)	8.90 (7.30-11.20)	11.50 (7.25-16.45)	0.048
HOMA-IR	1.97 (1.52-2.31)	2.43 (1.59-3.53)	0.030

Mann-Whitney test. * Fisher's exact test . **Chi-square test. Data are expressed median (25%-75%). SLE, Systemic Lupus Erythematosus; BMI, Body mass index; WC, Waist Circunference; HDL, High-density lipoprotein; LDL, Low-density lipoprotein; HOMA-IR, Homeostasis Model of Assessment Insulin Resistance.

Table 4: Inflammatory biomarkers in active and inactive SLE patients

Parameters	Inactive SLE (n = 38)	Active SLE (n = 36)	P value
C-reactive protein (mg/L)	1.155 (0.675-3.120)	2.610 (0.900-7.200)	0.030
α_1 acid glycoprotein	99.5 (76.0-128.0)	108.0 (83.5-144.5)	0.305
Adiponectin (µg/mL)	5.96 (4.07-9.15)	5.73 (4.26-7.57)	0.798
IL-6 (pg/mL)	8.20 (5.20-13.25)	8.60 (3.90-18.50)	0.851
IL-1 (pg/mL)	2.00 (2.00-2.00)	2.00 (2.00-2.00)	1.000
TNF-α (pg/mL)	2.00 (2.00-2.00)	2.00 (2.00-2.00)	0.443

Mann-Whitney test. Data are expressed median (25%-75%). IL-1, interleukin-1; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α

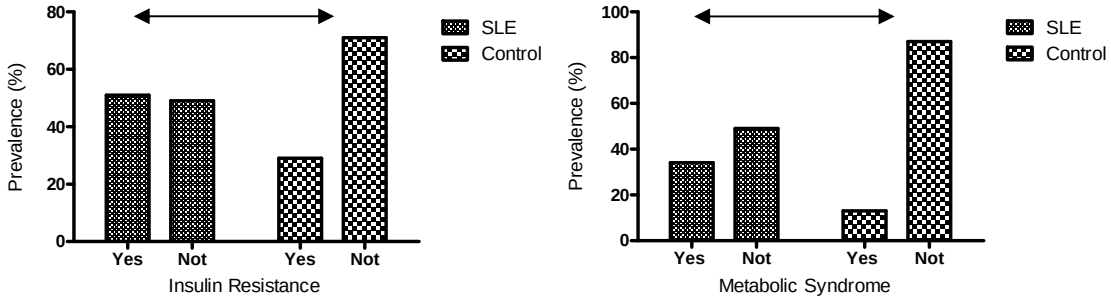

Figure 1: Prevalence of Insulin resistance and Metabolic Syndrome in Southern Brazilian patients with systemic lupus erythematosus and control subjects. Chi-square test. *OR=2.556 (CI 95%: 1.413-4.621, p=0.0017). **OR=4.644 (CI 95%: 2.240-9.625, p<0.0001).

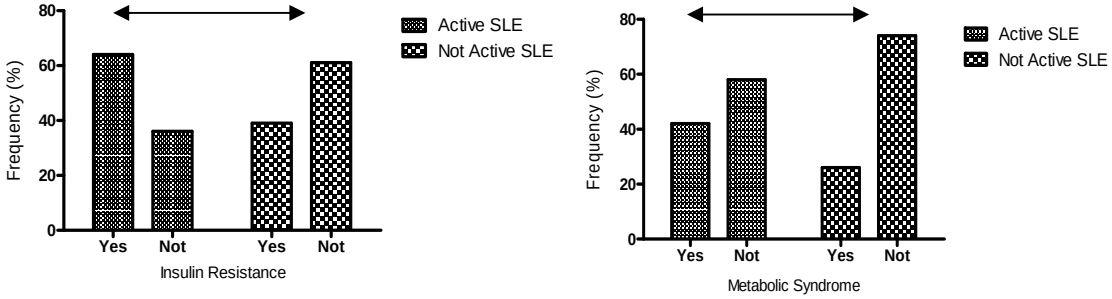

Figure 2: Frequency of Insulin resistance and Metabolic Syndrome in Southern Brazilian with systemic lupus erythemathosus (SLE) active or inactive. Chi-square test. *OR: 2.781 (IC 95%: 1.568-4.932, p=0.0004); **OR: 2.061 (IC 95%: 1.133-3.748, p=0.0169).

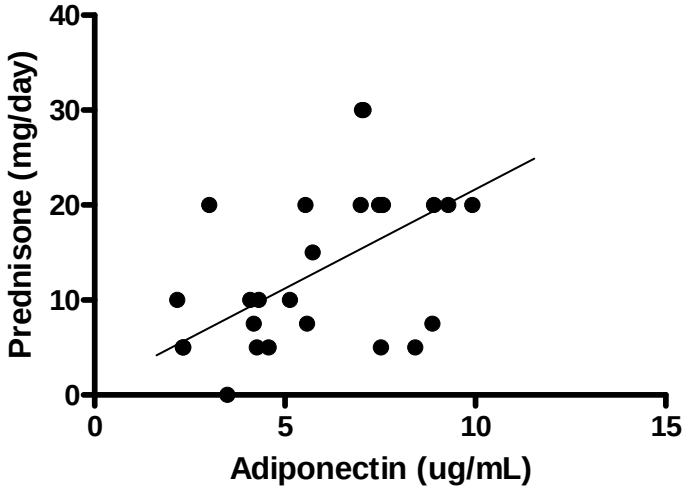

Figure 3: Spearman`s correlation between serum adiponectin levels and daily prednisone doses in patients Southern Brazilian with active systemic lupus erythematosus (r=0.437, p=0.018).

4.2. **ARTIGO 2:** INFLUENCE OF TH1/TH2/TH17 CYTOKINES LINEAGE, NITRIC OXIDE, OXIDATIVE STRESS, AND INSULIN RESISTANCE ON BLOOD PRESSURE IN ACTIVE AND INACTIVE SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS WITHOUT NEPHRITIS

Hypertension in lupus.

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ABSTRACT

Background: Hypertension is a major risk factor for cardiovascular and renal disease and is highly prevalent in women with SLE. The objectives of this study were to investigate several factors, which influence hypertension in patients with active (AT) and non-active (NAT) systemic lupus erythematosus (SLE) without renal damage. **Methods:** 102 healthy individuals, 70 NAT and 53 AT SLE patients without renal disease were selected. There were evaluated TH1/TH2/Th17 cytokines lineage, other proinflammatory cytokines, nitric oxide (NO), insulin resistance, and oxidative stress. Comparisons between groups were done using the Kruskal-Wallis test with post test of Dunns. Correlations were evaluated by Spearman's rank correlation. SLE AT patients presented higher probability to develop hypertension when compared to controls ($p<0.0001$, OR:4.101, CI 95%: 2.22-7.58) and also when compared to NAT SLE patients ($p=0.0016$, OR: 2.510 CI 95%: 1.408-4.473). SLE patients showed lower tumor necrosis factor alpha (TNF-α) ($p<0.05$) and interleukin (IL) 4 levels ($p< 0.0001$) and higher IL-6 ($p<0.0001$), IL-10 ($p< 0.01$), IL-17 ($p< 0.0001$), and interferon gamma (IFN-γ) levels ($p< 0.05$) than the control group. SLE patients had higher IFN-γ/IL4 ratio ($p<0.01$) than the control group, whereas AT SLE patients showed higher IL-12/IL-4 ratio ($p<0.05$) than controls and NAT patients. SLE AT and NAT patients presented higher hydroperoxydes

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levels ($p<0.05$, $p< 0.0001$, respectively) than controls, whereas serum protein oxidation levels were significantly higher in AT SLE patients compared with the control group and also in relation to NAT SLE patients ($p<0.01$, $p<0.05$, respectively). Total antioxidant capacity was significantly lower in NAT SLE than in the control group ($p<0.0001$). Serum nitric oxide metabolites levels were significantly higher in SLE patients in relation to the control group ($p<0.0001$). Systolic and diastolic blood pressure were directly correlated with serum insulin levels and HOMA-IR. On the other hand, systolic and diastolic blood pressure were inversely correlated with TRAP. In the meantime, nitric oxide had an inverse correlation with IFN-γ/IL-10 ratio and IFN-γ/IL-4 ratio. The present study showed that patients with AT SLE had more probability to develop hypertension than control subjects and patients with NAT SLE and this is due to increased Th_1/Th_2 ratio, pró-inflammatory and oxidative stress.

Key Indexing Terms: systemic lupus erythematosus, hypertension, cytokines, oxidative stress, nitric oxide, insulin resistance.

BACKGROUND

Systemic lupus erythematosus (SLE) is a systemic chronic inflammatory disorder characterized by a wide range of immunological hyperactivity, autoantibody production and multi-organ damages [1]. Hypertension is a major risk factor for cardiovascular and renal disease and is highly prevalent in women with SLE[2]. Elevated blood pressure (BP) results from environmental, genetic, and immunological factors and from the interactions among these factors [2,3]. The importance of the kidneys in the long-term control of blood pressure and the pathogenesis of hypertension are well understood and it is estimated that almost 50% of patients with SLE is affected by glomerulonephritis [2]. However, SLE is a risk factor for hypertension in humans and can occur independently of nephritis [4].

It has been suggested that chronic inflammatory process and higher TH_1 cytokines lineage (interferon gamma, IFN-γ; tumor necrosis factor alpha, TNF-α, etc) and its predominance over TH_2 cytokines lineage (interleukin 4, IL-4; interleukin 10, IL-10) can contribute to increasing blood pressure [3]. Moreover, in humans, blood pressure directly correlates with circulating inflammatory cytokines such as TNF-α, interleukin 6 (IL-6), and also with C-reactive protein (CRP) [5]. In the meantime, interleukin 17 (IL-17), a cytokine that acts independently of TH_1 and TH_2 cytokines lineage, has also been considered an important mediator of hypertension [3].

Systemic chronic inflammation has been proposed to have a prominent role in the pathogenesis of insulin resistance and MS [6]. Elevated serum concentration of key inflammatory cytokines [7] and glucocorticoids [8] use are associated with development of insulin resistance [7] and insulin resistance and hyperinsulinemia could also lead to hypertension [9].

Several studies suggest that oxidative stress is important in the pathogenesis of SLE [1,10] and is also recognized as an important factor in the pathogenesis of hypertension [3]. The mechanism by which oxidative stress promotes hypertension is related to vascular dysfunction, renal injury, and increased sodium reabsorption [2].

Besides the aforementioned factors, it is conceivable that disease activity could also have a role in SLE hypertension. However, to date, we are not aware of any study, which have analyzed this issue or have investigated all these factors concomitantly. Therefore, the aim of this study was to investigate several factors, including cytokines profile, oxidative stress and

insulin resistance, which influence hypertension in patients with active and non-active SLE without renal impairment.

SUBJECTS AND METHODS

Subjects

The study included 225 subjects (206 females and 19 males). One hundred and two healthy individuals (control group) were selected among blood donors of the University Hospital and one hundred and twenty three patients with SLE without renal disease (70 inactive SLE and 53 active SLE) were selected from among the ambulatory of Rheumatology of the University Hospital of Londrina, Paraná, Brazil, to participate in the study. They were paired by sex, age, ethnicity, body mass index (BMI), and waist circumference (WC). Systemic Lupus Erythematosus was diagnosed using the American College of Rheumatology (ACR) 1997 revised criteria [11]. Patients with SLE were also divided in two groups: with active disease (n=53) or inactive disease (n=70). The following parameters were used to classify SLE as active: SLE Disease Activity Index (SLEDAI) score ≥ 6, and/or decreased C3 (<90mg/dL) and/or decreased C4 complement (< 10 mg/dL), and/or positive anti-dsDNA (titre ≥ 1/10) [10, 12, 13]. Hypertension diagnose was considered when blood pressure ≥ 130/85 mmHg or when patients were in current use of antihypertensive medication.

Information on lifestyle factors and medical history were obtained at clinical evaluation. Disease duration, organ involvement, values of C3 and C4 complement, anti-double-stranded DNA (anti-dsDNA), and non-steroid anti-inflammatory drugs, corticosteroids, antimalarials, oral contraceptives, and antihypertensive medications were recorded for each patient. All patients were receiving prednisone at the time of inclusion, thus predinisone-equivalent calculation was not required. They had been taking the same prednisone dose at least for the past 4 months. No patient had clinical and laboratorial evidence of SLE nephritis. Nutritional status of patients was similar to that of the control group. None of the subjects were receiving a specific diet. The individuals of both groups did not drink alcohol regularly. None of the participants in the study presented heart, thyroid, renal, hepatic, gastrointestinal or oncological diseases, and none were receiving estrogen replacement therapy, drugs for hyperlipidemia, hyperglycemia or antioxidant supplements. Patients who were taking antihypertensive drugs were not excluded for ethical reasons and were allowed to continue taking the same dose of the drugs. This

study was conducted according to the guidelines laid down in the Declaration of Helsinki and the Ethical Committee of the University of Londrina, Paraná, Brazil approved all procedures involving human subjects and patients. Written informed consent was obtained from all subjects/patients.

Anthropometric and Blood Pressure Measurements

Body weight was measured to the nearest 0,1 kg by using an electronic scale, with individuals wearing light clothing, but no shoes, in the morning.; height was measured to the nearest 0,1 cm by using a stadiometer. Body mass index was calculated as weight (kg) divided by height (m) squared. Three blood pressure measurements taken with a minute interval between them after the subject had been seated were recorded. The mean of these measurements was used in the analysis [14].

Biochemical and Immunological Biomarkers

After fasting for 12 hours, the patients underwent the following laboratory blood analysis: uric acid and creatinine were evaluated by a biochemical auto-analyzer (Dimension Dade AR Dade Behring, Deerfield, IL, USA), using Dade Behring® kits; Plasma insulin and homocysteine level were determined by chemiluminescence microparticule immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA). The homeostasis model assessment insulin resistance (HOMA-IR) was used as a surrogate measurement of insulin sensitivity [15]. HOMA-IR = insulin fasting (μU/ml) X glucose fasting (nmol/L) / 22.5. Insulin resistance was considered when HOMA-IR $\geq$ 2.114 [16].

Serum complement factors C3 and C4 levels and hsCRP (highly sensitive CRP) were measured using a nephelometric assay (Behring Nephelometer II, Dade Behring, Marburg, Germany). Anti-double-stranded DNA (anti-dsDNA) antibodies were determined by immunoflurescence using *Crithidia lucilliae* kinetoplast assay (Obelis S/A, Brussels, Belgium).

TNF-α, IL-4, interleukin 6 (IL-6), IL-10, interleukin 12 (IL-12), IL-17, IFN-γ and adiponectin levels were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a commercial immunoassay ELISA (Ready-Set Go! Set, e-Bioscience, San Diego, California, USA).

Oxidative Stress Measurements

Samples for evaluating oxidative stress and total antioxidant capacity were performed with EDTA as anticoagulant and antioxidant. All samples were centrifuged at 3.000 rpm for 15 minutes and plasma aliquots stored at -70ºC until assayed.

Total radical-trapping antioxidant parameter (TRAP)

The TRAP was determined as reported by Reppeto et al [17]. This method detects hydrosoluble and/or liposoluble plasma antioxidants by measuring the chemiluminescence inhibition time induced by 2,2-azobis (2-amidinopropane). The system was calibrated with the vitamin E analog TROLOX, and the values of TRAP are expressed in equivalent of μM Trolox/mg uric acid.

Tert-butyl hydroperoxide-initiated chemiluminescence (CL-LOOH)

CL-LOOH in plasma was evaluated as described previously by Flecha et al [18]. The results are expressed in counts per minute (cpm).

Determination of Advanced Oxidation Protein Products (AOPP)

AOPP was determined in the plasma using the semiautomated method described by Witko-Sarsat et al [19]. AOPP concentrations were expressed as micromoles per liter (umol/L) of chloramines-T equivalents.

Nitric Oxide (NO)

Serum NO metabolite (NOx) levels were assessed by nitrite (NO_2^-) and nitrate (NO_3^-) concentration according to the Griess reaction, supplemented by the reduction of nitrate to nitrite with cadmium [20].

Statistical analysis

Distribution of sex, race, and smoking was analyzed with Fisher's exact test or chi-square test. Comparisons between groups were done using the Kruskal-Wallis test with post test of Dunns and data were expressed as the median (25-75 percentiles). Correlations were evaluated by Spearman's rank correlation. The results were considered significant when $p<0.05$. A statistical analysis program (Graph Pad Prism version 4.0) was used for evaluations.

RESULTS

Clinical and laboratory characteristics of patients with non active (NAT) and active (AT) SLE are shown in table 1. The groups did not differ in relation to the frequency of patients using antimalarials or antihypertensive drugs. However, the number of patients who were taking prednisone was significantly higher in the AT group of patients (p=0.0363), whereas the number of patients who were taking immunosuppressive drugs were higher in the NAT group (p=0.0077, respectively).

There were no differences in the control group, NAT and AT SLE with regard to age, gender, ethnicity, tabagism, and BMI, creatinine, and uric acid levels. Non active SLE and AT SLE had significant increase in diastolic blood pressure ($p<0.05$ and $p<0.0001$, respectively), insulin levels ($p<0.0001$) and HOMA-IR (($p<0.0001$) when compared to control subjects. In addition, AT SLE showed significant increase in systolic blood pressure ($p<0.01$) and CRP ($p<0.0001$) in relation to the control group. There were no significant differences between NAT and ACT SLE patients (Table 2).

Active SLE patients presented higher prevalence of hypertension when compared to controls ($p<0.0001$, OR:4.101, CI 95%: 2.22-7.58) and also when compared to NAT SLE patients (p=0.0016, OR: 2.510 CI 95%: 1.408-4.473) (Figure 1).

In relation to cytokine profile, NAT and AT SLE patients showed lower ($p<0.05$) TNF-α and higher IL-12 ($p<0.0001$) levels than the control group. On the other hand, the Th_1 cytokine profile NAT and AT SLE patients demonstrated higher IFN-γ ($p<0.05$) levels than the control group. In relation to Th_2 cytokine profile, NAT and AT SLE patients showed lower ($p<0.0001$) IL-4 levels than the control group. On the other hand, NAT and AT SLE patients demonstrated higher IL-10 and IL-6 levels than the control group ($p<0.01$, $p<0.05$, and $p<0,0001$, respectively). NAT and AT SLE patients had higher IFN-γ/IL-4 ratio than the control group ($p<0.01$ and $p<0.0001$, respectively), whereas AT SLE patients showed higher ($p<0.05$) IL-12/IL-4 ratio than controls and NAT SLE patients. In the meantime, AT and NAT SLE patients showed higher ($p<0.0001$) levels of IL-17, a cytokine that develops independently of the TH_1 and TH_2 lineages, in relation to the control group (Table 3). Non active and AT SLE patients also had lower ($p<0.05$) adiponectin levels than the control group (Figure 2).

With regard to redox state assessment, patients with NAT SLE and AT SLE presented higher hydroperoxydes levels than controls ($p<0.0001$; $p<0.05$, respectively), whereas serum

AOPP levels were significantly higher in AT SLE patients in relation to the control group ($p<0.01$) and also in relation to NAT SLE patients ($p<0.05$). On the other hand, TRAP corrected by uric acid levels was significantly lower in non AT SLE ($p<0.0001$) than in the control group (Table 4). Serum NOx levels were significantly higher in NAT SLE and AT SLE in relation to the control group ($p<0.0001$ and $p<0.05$, respectively) (Figure 3).

Spearman's correlation was performed in SLE patients. Systolic and diastolic blood pressure were directly correlated with serum insulin levels ($r=0.2069$, $p=0.0293$; $r=0.2479$, $p=0.0087$, respectively) and HOMA-IR ($r=0.2633$, $p=0.0055$; $r=0.2733$, $p=0.0039$, respectively). On the other hand, systolic and diastolic blood pressure were inversely correlated with TRAP ($r= -0.2946$, $p=0.0031$; $r= -0.3549$, $p=0.003$, respectively). In the meantime, nitric oxide had an inverse correlation with IFN-γ/IL-10 ratio and IFN-γ/IL-4 ratio ($r= -0.3150$, $p=0.0259$; $r= - 0.3949$; $p=0.0024$) (figure 4).

DISCUSSION

The main finding of the present study is that patients with AT SLE have 4.101 and 2.510 more probability to develop hypertension than control subjects and patients with NAT SLE, respectively. In addition, this study showed that increased Th_1/Th_2 (IFN-γ/IL-4) ratio and increased oxidative stress (protein oxidation) are the factors, which influence hypertension when patients with AT SLE are compared with patients with NAT SLE without renal damage.

Hypertension is a major risk factor for the progression of renal, vascular, and cardiac disease. Our data are in accordance with previous studies, which also demonstrated high hypertension prevalence in patients with SLE [21, 22]. Nevertheless, there are no studies, to date, that have related hypertension with disease activity.

The role of oxidative stress in SLE hypertension

The over production of reactive species or impaired scavenging promote oxidative stress and is recognized as important in the pathogenesis of hypertension [2]. The evaluation of redox state showed that patients with SLE had decreased antioxidant defences and increased oxidative stress and serum NOx levels, and that AT SLE patients had higher protein oxidative stress than NAT SLE patients. We have previously verified that AT or NAT SLE patients did not present increase oxidative stress, however this finding was attributed to the increased glucocorticoid consumption in AT SLE patients [10]. Differently, in the present

study, there was no difference in glucocorticoid consumption in the SLE groups, what could explain the relation found between higher oxidative stress and disease activity. In addition, total antioxidant capacity showed an inverse correlation with blood pressure in patients with SLE. Thus, it is conceivable to suggest that decrease in antioxidant mechanisms may be an important link between the chronic inflammatory process and the development of hypertension in SLE patients.

The role of Th_1/Th_2/Th17 and pro-inflammatory cytokines lineage in SLE hypertension

The role of Th_1, Th_2 and Th_{17} cytokines lineage was assessed in this study and it could be verified that NAT and AT SLE patients presented, in general, higher cytokine levels in all aforementioned lineages when compared to control subjects. In addition, higher IFN-γ/IL-4 ratio showed that Th1 predominated over Th2 cytokines lineage. Moreover, increase in IL-12/IL-4 ratio showed a pro inflammatory state in patients with AT SLE compared with NAT SLE and controls. Our data are in agreement with a previous study, which has also shown the increase in both, Th_1 and Th_2 lineages, in SLE patients in relation to controls, and that Th_1 profile predominated over Th_2 in active SLE patients [23]. It has been demonstrated that when Th_1 lineage predominates, it can contribute to increased blood pressure [3].

It has been suggested that is quite likely that TNF-α and IL-6 contribute by creating a cytokine milieu that promotes hypertension [3]; TNF-α antagonist etanercept prevents hypertension and vascular dysfuntion in angiotensin II-induced hypertension [24] and others authors have shown that it prevents hypertension in frutose-fed rats [25]. In addition, IL-6 has also been implicated in angiotensin II-induced hypertension [26]. In the present study, TNF-α levels decreased and IL-6 level increased in SLE patients when compared with the control group. This apparent paradox could be explained by some factors. First, serum TNF-α levels obtained does not represent the actual concentration of this cytokine locally produced in the site of inflammation [27]. Second, the detectable serum level of TNF-α does not take into account the membrane-bound form of TNF-α, which is up regulated in many cells, including endothelial cells and leukocytes, during an inflammatory process [28]. In addition, Gomez et al. [23] similarly to the results found in the present study, verified that TNF-α was decreased in relation to disease activity, and thus could have an immunosuppressive role in SLE.

In the meantime, serum IL-17 levels were also elevated in patients with SLE, independently of disease activity. IL-17 is a novel cytokine produced by Th_{17} cells, cytotoxic T cells, mast cells, neutrophils and natural killer T cells [29]. IL-17 has been shown to induce

chemokines and adhesion molecules in tissues that promote tissue accumulation of other inflammatory cells. IL-17 may act synergically with other cytokines, such as TNF-α, to modulate inflammatory response and both are important to contribute to hypertensive phenotype [30]. In the present study, increased IL-17 and IL-6 levels reinforces the hypothesis of synergic action between these cytokines, but not with TNF-α. Harrison et al. [3] proposed that T cells residing in the perivascular fat release cytokines such as IL-17, that diffuse to the adjacent vascular smooth muscle cells where they enhance superoxide production, reduce endothelium-dependent vasodilatation and promote vasoconstriction.

The link between NO and adiponectin and Th_1/Th_2 cytokines lineage in SLE hypertension

Numerous epidemiological studies based on different ethnic groups have identified an adiponectin deficiency as an independent risk factor for endothelial dysfunction, hypertension, coronary heart disease, myocardial infarction, and other cardiovascular complications. Adiponectin has anti-inflammatory effects and augments blood flow by enhancing nitric oxide production and activating endothelial nitric oxide synthase (eNOS) [31]. NO plays a major role in regulating blood pressure, and its deficient bioavailability is an important component of hypertension [32]. NO has been considered the principal mediator of vasodilatation caused by endothelial cells. NO is synthesized in endothelial cells by endothelial nitric oxide synthase (eNOS) activity, and is responsible for vasodilatation and for the maintenance of endothelial function; eNOS is expressed constitutively and synthesizes NO in only small amounts under basal conditions. However, NO production may also be stimulated by an increase in inducible nitric oxide synthase (iNOS) expression provoked, for instance, by oxidative stress or pro-inflammatory cytokines. Therefore, iNOS derived NO mediates the inflammatory response and has been shown to cause vascular dysfunction in a number of experimental models [33].

The results of the present study showed increase in NOx levels and oxidative stress, decrease in adiponectin levels and higher probability to present hypertension in SLE patients in relation to control subjects. It is conceivable that increased NOx level is due to iNOS induction by oxidative stress. However, there is a higher NO consumption in oxidative stress reactions, with consequent decrease in NO bioavailability [34]. Oxidative redox stress results in impaired endothelium-dependent vasodilation with quenching of endothelial nitric oxide and allows the endothelium to become a net producer of ROS specifically superoxide as the eNOS enzyme uncouples to produce superoxide instead of eNOS [35]. Hypertensive subjects

have increased generation of reactive oxygen species (ROS), which scavenge NO, thereby reducing NO bioavailability [32]. In addition, our data showed a direct correlation between nitric oxide and Th1/Th2 cytokines lineage ratio, suggesting a role of the immunological system in the control of NOx levels.

The role of insulin resistance in SLE hypertension

Elevated serum concentrations of key inflammatory cytokines, such as IL-6 and TNF-α, are associated with development of IR [7] and hypertension [3]. Hyperinsulinemia and insulin resistance could lead to sodium renal tubular absorption, which in turn could provoke hypertension [9]. In the present study, patients with SLE had higher insulin levels and HOMA-IR than control subjects. In addition, there was a direct correlation between blood pressure and serum insulin levels and HOMA-IR. Of note, rosiglitazone, a thiazolidinedione class of drug used to regulate glucose levels in insulin-resistant patients, have shown to decrease blood pressure in a female mouse model of SLE [36].

CONCLUSION

The data of the present study suggest the complex interaction of cytokines, nitric oxide, oxidative stress and insulin resistance on blood pressure changes in SLE patients. In addition, the main factors, which influenced the higher frequency of hypertension in AT SLE patients than in NAT SLE patients were increased Th_1/Th_2 ratio and increased oxidative stress. The present study also showed that patients with AT SLE have more probability to develop hypertension than control subjects and patients with NAT SLE. The risk to develop hypertension in SLE patients is related to immune system involvement, oxidative stress and insulin resistance. At last, this study helps the understanding of the complex physiopathology of hypertension in SLE patients and reinforces the possibility of new potential therapeutic targets in the treatment of hypertension in systemic lupus erythematosus.

Acknowledgments

This work was supported by the University of Londrina Research Funds (FAEPE).

Abbreviations

Anti-dsDNA: Anti-double-stranded DNA; AOPP: Determination of Advanced Oxidation Protein Products; AT: active; BMI: body mass index; CL-LOOH: Tert-butyl hydroperoxide-

initiated chemiluminescence; Cont: control; DBP: Diastolic Blood Pressure; eNOS: endothelial nitric oxide synthase; HOMA-IR: homeostasis model assessment insulin resistance; HsCRP: highly sensitive CRP; IFN-γ: interferon gamma; IL: interleukin; IL-4: Interleukin 4; IL-6: Interleukin 6; IL-10: Interleukin 10; IL-12: Interleukin 12; IL-17: Interleukin 17; iNOS: inducible nitric oxide synthase; NAT: non-active; NO: Nitric Oxide; Nox: nitric oxide metabolites; ROS: reactive oxygen species; SBP: Systolic Blood Pressure; SLE: systemic lupus erythematosus; SLEDAI: systemic lupus erythematosus disease activity index; TNF-α: tumor necrosis factor alpha; TRAP: Total Radical-Trapping Antioxidant Parameter; WC: waist circunference

Competing interests

The authors declare that they have no competing interests.

Authors´ Contribution

The authors' responsibilities were as follows: MABL and ANCS collected the data, designed the study, interpreted the results and wrote the manuscript; HKM, SDB, and EMVR performed cytokine analysis; TMVI performed clinical evaluation of the patients, CP and SRO performed oxidative stress analysis; RC interpreted the results and wrote the manuscript; and ID developed the hypothesis tested in the study, designed the study, interpreted the results and wrote the manuscript. None of the authors had any conflict of interest in relation to this study. All authors read and approved the final manuscript.

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Figure Legend

Figure 1: Frequency of hypertension in healthy subject (Cont) and in patients with active (AT) or non active (NAT) systemic lupus erythematosus (SLE). Chi-square test. **p<0.0001 (OR: 4.101, IC 95%: 2.22-7.58), SLE AT vs Cont; *p=0.0016 (OR: 2.510, IC 95%: 1.408-4.473) SLE NAT vs SLE AT.

Figure 2: Plasma adiponectin levels in healthy subject (Cont) and in patients with active (AT) or non active (NAT) systemic lupus erythematosus (SLE). Chi-square test. *p<0.05 when compared with the control group.

Figure 3: Serum nitric oxide metabolites (NOx) levels in healthy subject (Cont) and in patients with active (AT) or non active (NAT) systemic lupus erythematosus (SLE). Chi-square test. *p<0.0001 when compared with the control group.

Figure 4: Spearman' correlation in patients with systemic lupus erythematosus. PAS: Systolic Blood Pressure; PAD: Diastolic Blood Pressure; HOMA-IR: homeostasis model assessment insulin resistance; TRAP: Total radical-trapping antioxidant parameter; IFN-γ: interferon gamma; ; IL10: interleukin 10; IL-4: interleukin 4

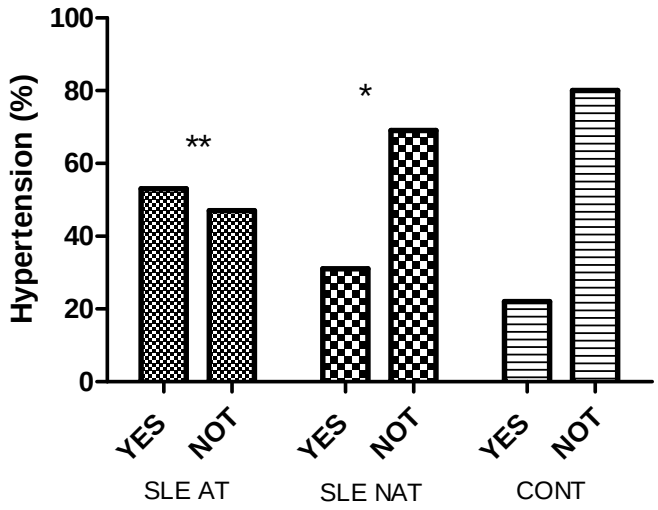
Hypertension (%)
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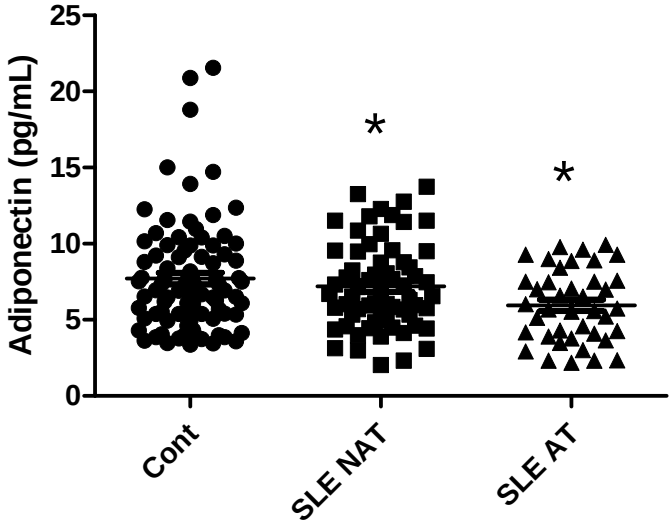
Figure 1:



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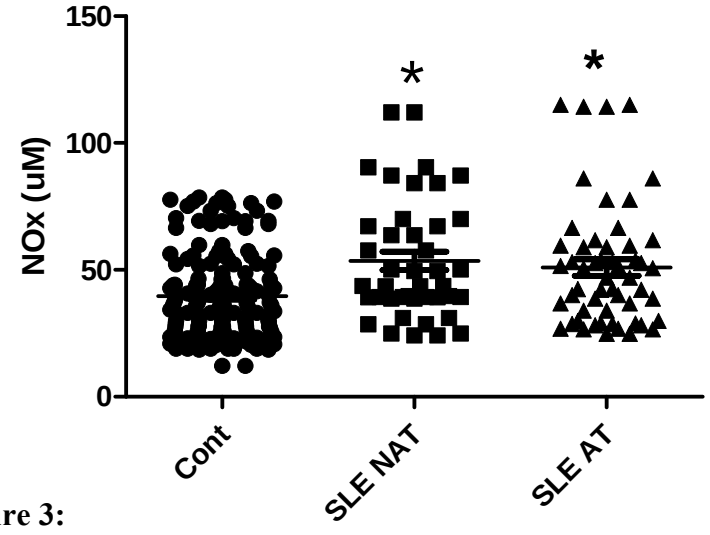
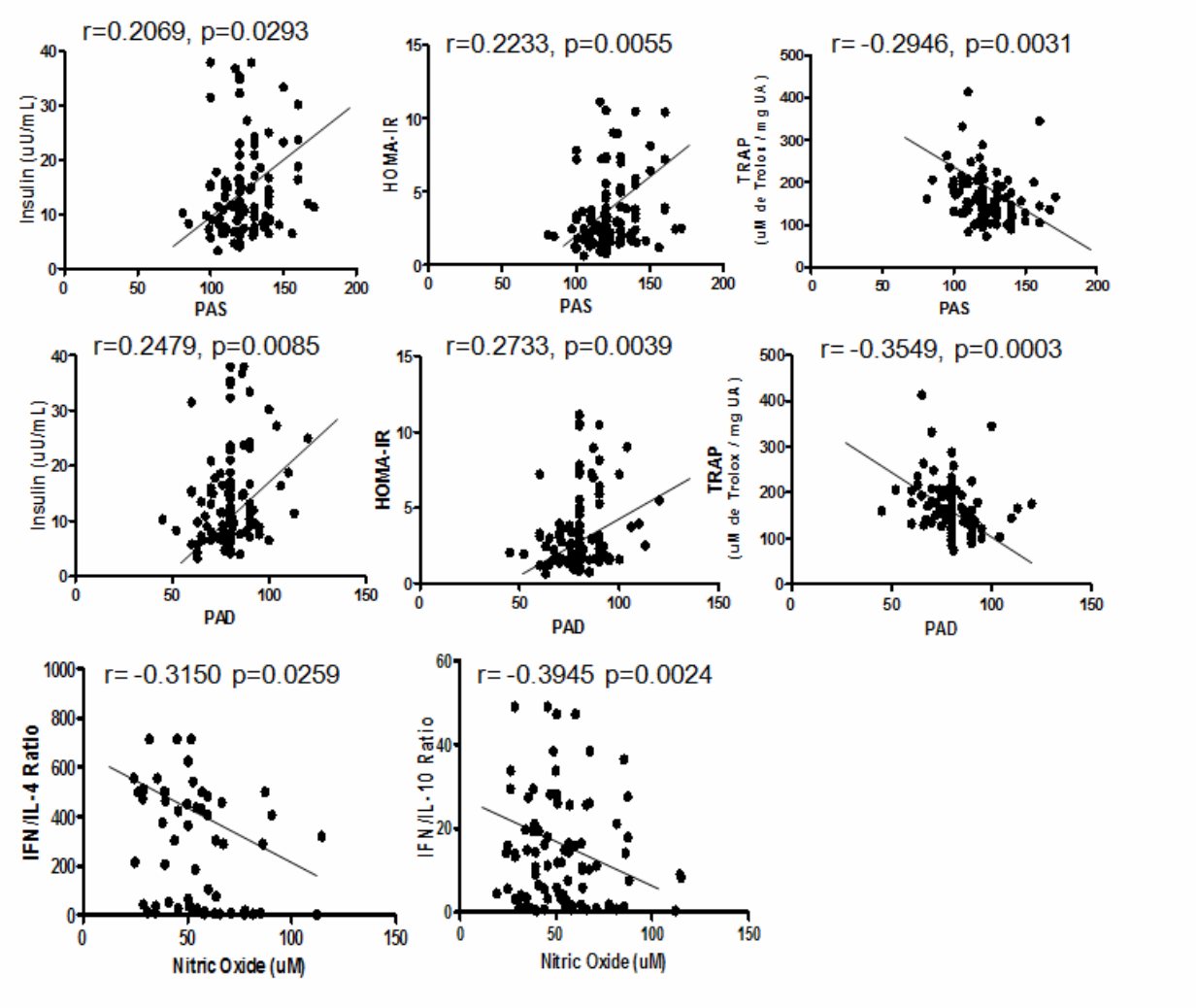


Figure 3:


r=0.2069, p=0.0293
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PAS
r=0.2233, p=0.0055
HOMA-IR
PAS
r= -0.2946, p=0.0031
TRAP
(uM de Trolox / mg UA)
PAS
r=0.2479, p=0.0085
Insulin (uU/mL)
PAD
r=0.2733, p=0.0039
HOMA-IR
PAD
r= -0.3549, p=0.0003
TRAP
(uM de Trolox / mg UA)
PAD
r= -0.3150 p=0.0259
IFN/IL-4 Ratio
Nitric Oxide (uM)
r= -0.3945 p=0.0024
IFN/IL-10 Ratio
Nitric Oxide (uM)

Figure 4:

Table 1: Clinical and laboratory characteristics of patients with non active (NAT) and active (AT) systemic lupus erythematosus (SLE).

	NAT SLE (n=70)	AT SLE (n=53)	P
Disease Duration (years)*	9.0 (4.0-12.0)	10.0 (6.0-15.7)	0.2260
SLEDAI Score ≥ 6	0	13	----
Anti-dsDNA Positive	0	22	----
C3 (<90mg/dL)	0	13	----
C4 (<10 mg/dL)	0	10	
Therapy			
Prednisone (Y/N)	64/6	53/0	0.0363
Prednisone (mg/day)*	10.0 (5.0-20.0)	15.0 (5.6-20.0)	0.1322
Antimalarials (Y/N)	38/32	36/17	0.1260
Current Immunosuppressive (Y/N)	25/45	16/37	0.0077
Anti-Hypertensive drugs (Y/N)	15/55	18/35	0.1203

Chi-square test. *Mann-Whitney test. Data are expressed as median (25-75%).

SLEDAI: systemic lupus erythematosus disease activity index; Y: yes; N: no

Table 2: Clinical and laboratory characteristics of controls (Cont) and patients with non active (NAT) and active (AT) systemic lupus erythematosus (SLE).

Table 2: Clinical and laboratory characteristics of controls (Cont) and patients with non active (NAT) and active (AT) systemic lupus erythematosus (SLE).

	Cont (n=102)	NAT SLE (n=70)	AT SLE (n=53)	C vs NAT SLE	C vs AT SLE	NAT SLE vs AT SLE
Gender (F/M)	92/10	66/4	48/5	NS	NS	NS
Caucasian/not Caucasian	81/21	50/20	37/16	NS	NS	NS
Smoking/ Non smoking	4/98	2/68	1/52	NS	NS	NS
Age (years)	40.0 (32.0-46.0)	39.5 (31.0-52.5)	43.0 (30.5-51.0)	NS	NS	NS
BMI (kg/m^2)	26.0 (24.1-29.4)	26.4 (23.5-30.9)	28.8 (26.1-32.8)	NS	NS	NS
WC (cm)	93.0 (87.5-101.5)	94.0 (85.0-104.5)	96.8 (87.3-107.3)	NS	NS	NS
SBP (mmHg)	111.0 (101.0-126.0)	120.0 (110.0-127.8)	120.0 (116.0-135.5)	NS	<0.01	NS
DBP (mmHg)	71.0 (65.0-80.0)	80.0 (70.0-80.0)	80.0 (75.0-90.0)	<0.05	<0.0001	NS
Creatinine	0.75 (0.66-0.90)	0.75 (0.66-0.90)	0.69 (0.64-0.83)	NS	NS	NS
Uric acid (mg/dL)	3.87 (3.33-4.73)	4.24 (3.31-4.95)	4.20 (3.35-4.93)	NS	NS	NS
CRP (mg/dL)	1.99 (0.88-3.81)	2.17 (0.67-6.69)	4.17 (1.10-7.22)	NS	<0.05	NS
Insulin (μU/mL)	7.60 (5.05-11.60)	11.30 (8.10-15.85)	12.70 (8.00-20.95)	<0.0001	<0.0001	NS
HOMA-IR	1.59 (1.02-2.68)	2.40 (1.60-3.52)	2.75 (1.68-4.95)	<0.0001	<0.0001	NS

Kruskal-Wallis test with post test of Dunns. Data are expressed as median (25-75%). BMI: body mass index; WC: waist circunference; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; CRP: C-reactive protein; HOMA-IR: homeostasis model assessment insulin resistance.

Table 3: Cytokine profile of controls (Cont) and patients with non active (NAT) and active (AT) systemic lupus erythematosus (SLE).

	Cont (n=102)	SLE NAT (n=70)	SLE AT (n=53)	C vs SLE NAT	C vs SLE AT	SLE NAT vs SLE AT
TNF-α (pg/mL)	2.00 (2.00-10.40)	2.00 (2.00-4.03)	2.00 (2.00-3.40)	<0.05	<0.05	NS
IL-6 (pg/mL)	1.00 (1.00-3.35)	5.35 (1.93-11.65)	4.30 (2.35-9.85)	<0.0001	<0.0001	NS
IL-17 (pg/mL)	2.00 (2.00-2.00)	3.90 (2.00-7.25)	5.95 (2.00-7.95)	<0.0001	<0.0001	NS
IL-12 (pg/mL)	2.10 (2.00-2.00)	2.10 (2.00-7.15)	2.0 (2.00-4.00)	<0.0001	<0.0001	NS
IL-10 (pg/mL)	4.50 (3.30-6.20)	9.15 (1.40-18.73)	10.50 (1.00-26.30)	<0.01	<0.05	NS
IL-4 (pg/mL)	1.00 (0.70-11.0)	1.00 (0.70-2.10)	0.95 (0.70-3.60)	<0.0001	<0.0001	NS
IFN-γ (pg/mL)	38.25 (14.63-258.20)	242.20 (14.08-500.00)	225.60 (38.20-382.60)	<0.05	<0.05	NS
IL-12/IL-10	0.25 (0.16-2.42)	0.26 (0.19-2.12)	0.22 (0.11-2.82)	NS	NS	NS
IL-12/IL-4	3.00 (2.00-5.21)	2.93 (2.00-5.89)	4.71 (3.0-7.43)	NS	<0.05	<0.05
IFN/IL-10	14.62 (3.37-37.92)	13.36 (1.64-36.47)	14.87 (6.07-42.85)	NS	NS	NS
IFN/IL-4	31.90 (13.18-219.70)	304.60 (13.65-500.00)	322.20 (39.36-474.91)	<0.01	<0.0001	NS
TNF/IL-10	0.28 (0.14-2.0)	0.28 (0.11-2.00)	2.00 (0.07-2.00)	NS	NS	NS
TNF/IL-4	2.86 (2.00-4.75)	2.86 (2.00-4.39)	2.87 (2.70-4.75)	NS	NS	NS

Kruskal-Wallis test with post test of Dunns. Data are expressed median (25-75%). TNF-α: tumor necrosis factor-α; IL-6: interleukin 6,IL-17: interleukin 17; IL-12: interleukin 12; IL10: interleukin 10; IL-4: interleukin 4; IFN-γ: interferon gamma

	Cont	SLE NAT	SLE AT	C vs SLE NAT	C vs SLE AT	SLE NAT vs SLE AT
Hidroperoxides (cpm)	13690 (11090-17480)	19530 (12650-30810)	17810 (12260-24600)	<0.0001	<0.05	NS
AOPP (umol/L)	128.5 (94.9-166.2)	126.4 (102.5-156.1)	156.2 (119.4-201.6)	NS	<0.01	<0.05
TRAP (µM Trolox/mg UA)	181.5 (153.7-213.6)	146.9 (124.9-181.6)	161.0 (136.0-205.1)	<0.0001	NS	NS

Table 4: Oxidative stress and total antioxidant capacity of controls (Cont) and patients with non active (NAT) and active (AT) systemic lupus erythematosus (SLE).

Kruskal-Wallis test with post test of Dunns. Data are expressed median (25-75%). AOPP: Advanced Oxidation Protein Products; TRAP: Total radical-trapping antioxidant parameter

4.3 ARTICLE 3: RELATIONSHIP BETWEEN IRON METABOLISM, OXIDATIVE STRESS AND INSULIN RESISTANCE IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Ferritin, insulin, and oxidative stress in SLE

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ABSTRACT

Objective: The aim of the present study was to assess oxidative stress and iron metabolism in SLE patients with and without IR.

Methods: This study included 236 subjects (125 controls and 111 SLE patients). Patients with SLE were divided in two groups: with (n=72) or without (n=39) IR.

Results: SLE patients with IR showed higher advanced oxidation protein products **(**AOPP) (p = 0,030), and gamma-glutamyltransferase levels (p = 0,001), and lower sulfhydryl groups of proteins (p = 0.0002) and total radical-trapping antioxidant parameter (TRAP) corrected by uric acid (UA) levels (p = 0.04) when compared to SLE patients without IR. However, SLE patients with IR presented lower serum 8-isoprostanes (p = 0.05) and carbonyl protein levels (p = 0.04) when compared to SLE patients without IR. Serum ferritin levels were significantly higher in SLE patients (p = 0.0006) when compared to controls and SLE patients with IR presented higher serum ferritin levels (p = 0.01) compared to SLE patients without IR. Patients with SLE showed that IR was inversely correlated to TRAP/UA (r = -0.2724, p = 0.0008) and serum ferritin was positively correlated with AOPP (r = 0.2870, p = 0.004).

Conclusion: This study showed that IR increases oxidative stress in patients with SLE and that increased ferritin whatever its cause, the inflammatory process per se or

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hyperinsulinemia, can favor the redox process. Moreover, the preset data reinforce the need of measuring oxidative stress with several methodologies with different assumptions.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a systemic autoimmune disease characterized by multisystem organ involvement and by high titers of autoantibodies against several nuclear and cytoplasmatic antigens. SLE has a strong inflammatory component and consequently chronic over-production of reactive oxygen species (ROS) and reactive nitrogen species occurs (RNS). [1] Oxidative stress may contribute to immune-cell dysfunction, autoantigen production and autoantibody reactivity in SLE. [1] In autoimmune disease, oxidatively modified proteins can generate neo-epitopes from self-proteins, causing aggressive autoimmune attack. [2]

Systemic chronic inflammation and oxidative stress has been proposed to have a prominent role in the pathogenesis of insulin resistance (IR). [3-5] Several studies have demonstrated that SLE patients have more severe IR compared with the general population. [6-8]

Ferritin and iron homeostasis have also been implicated in the phatogenesis of many diseases, including autoimmune disease, insulin resistance and metabolic syndrome. [9-11] Elevated ferritin may interfere with hepatic insulin extraction leading to peripheral hyperinsulinemia and IR. It is known that liver-mediated insulin resistance is an early consequence of iron-dependent damage. [12-14] The main function of ferritin is to store intracellular iron in a compact, safe way, and make it available when necessary. This function is due to a very high iron binding capacity and its ferroxidase activity, which converts Fe^{++} to Fe^{+++}. [15] Several reports support a role of ferritin as a protectant against oxygen free radical-mediated damage. [15] However, observations that superoxide can mobilize iron from ferritin led to the suggestion that exposure to oxygen radicals may actually increase the pool of reactive (reduced) iron and exacerbate oxidant injury [16]. Moreover, iron catalyzes the formation of hydroxyl radicals, which are powerful prooxidants that attack cellular membrane lipids, proteins, and nucleic acids. [16] Nevertheless, controversies exist on the functional role of ferritin in these conditions, whether pro or antioxidant.

Therefore, ferritin increased levels are associated with chronic inflammatory process and IR and can be involved in the redox imbalance verified in these conditions. To date, relationship

between iron metabolism, insulin resistance and oxidative stress evaluated concomitantly has not been investigated in the scientific literature on SLE. Our hypothesis was that SLE patients with insulin resistance would have higher oxidative stress and ferritin levels than SLE patients without insulin resistance and that oxidative stress would be correlated with iron metabolism parameters and insulin resistance. Thus, the aim of the present study was to assess oxidative stress and iron metabolism in SLE patients with and without insulin resistance.

SUBJECTS AND METHODS

Subjects

The study included 236 subjects. One hundred and twenty five healthy individuals were selected from among blood donors of the University Hospital and 111 patients with SLE were selected from among the ambulatory of Rheumatology of the University Hospital of Londrina, Paraná, Brazil, to participate in the study. They were paired by sex, age, ethnicity, and body mass index (BMI). Systemic Lupus Erythematosus was diagnosed using the American College of Rheumatology (ACR) 1997 revised criteria. [17] The following parameters were used to classify SLE as active: SLE Disease Activity Index (SLEDAI) [18] score ≥ 6 [19], and/or decreased C3 (<90mg/dL) and/or decreased C4 complement (< 10 mg/dL), and/or positive anti-dsDNA (titre ≥ 1/10).[19]

Information on lifestyle factors and medical history were obtained at clinical evaluation. Disease duration, organ involvement, values of C3 and C4 complement, Anti-double-stranded DNA (anti-dsDNA), and non-steroid anti-inflammatory drugs, corticosteroids, antimalarials, oral contraceptives, and antihypertensive medications were recorded for each patient. All patients were receiving prednisone at the time of inclusion, thus predinisone-equivalent calculation was not required. They had been taking the same prednisone dose at least for the past 4 months. No patient with SLE presented proteinuria. Nutritional status of patients was similar to that of the control group. None of the subjects were receiving a specific diet. The individuals of both groups did not drink alcohol regularly. None of the participants in the study presented heart, thyroid, renal, hepatic, gastrointestinal or oncological diseases, and none were receiving estrogen replacement therapy, drugs for hyperglycemia, statins or antioxidant supplements**.** All patients gave written informed consent, and the study protocol

was fully approved by the Ethical Committee of the University of Londrina (Paraná, Brazil).

Anthropometric Measurements

Body weight was measured to the nearest 0,1 kg by using an electronic scale, with individuals wearing light clothing, but no shoes, in the morning.; height was measured to the nearest 0,1 cm by using a stadiometer. Body mass index was calculated as weight (kg) divided by height (m) squared.

Biochemical and Immunological Biomarkers

After fasting for 12 hours, the patients underwent the following laboratory blood analysis: glucose, iron, uric acid, gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were evaluated by a biochemical auto-analyzer (Dimension Dade AR Dade Behring, Deerfield, IL, USA), using Dade Behring® kits; plasma insulin, ferritin and homocysteine levels were determined by chemiluminescence microparticule immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA). The homeostasis model assessment (HOMA) was used as a surrogate measurement of insulin sensitivity [20].

HOMA for insulin resistance (HOMA-IR) = insulin fasting (μU/ml) X glucose fasting (nmol/L) / 22.5. Insulin resistance was considered when HOMA-IR ≥ 2.11 [21].

Serum complement factors C3 and C4 levels were measured using a nephelometric assay (Behring Nephelometer II, Dade Behring, Marburg, Germany). Anti-double-stranded DNA (anti-dsDNA) antibodies were determined by immunoflurescence using *Crithidia lucilliae* kinetoplast assay (Obelis S/A, Brussels, Belgium).

Oxidative Stress Measurements

Samples for evaluating oxidative stress and total antioxidant capacity were performed with EDTA as anticoagulant and antioxidant. All samples were centrifuged at 3.000 rpm for 15 minutes and plasma aliquots stored at -70ºC until assayed.

Tert-butyl hydroperoxide-initiated chemiluminescence (CL-LOOH)

CL-LOOH reflects lipid hydroperoxides originated from phospholipids, cholesterol esters, protein and free fat acid oxidation and decreased antioxidants levels, brought about by previous free radicals action mainly on plasma lipoprotein particles. This method is much more sensitive and less prone to artifact than others used in SLE patients. [19] CL-LOOH in plasma was evaluated as described previously by Flecha et al. [22] and reported previously by our group. [19] The results are expressed in counts per minute (cpm).

Determination of 8-Isoprostane

The plasma levels of free 8-isoprostanes F2 were quantified using a competitive immunoenzymatic kit (Cayman Chemical Company, Ann Arbor, Michigan, EUA) based on the activity of 8-isoprostane acetylcholinesterase conjugate. After alkaline hydrolysis of isoprostane esters in plasma samples, supernatants were added to the microplate reaction and the absorbance of the product formed from the reaction between thiocholine and 2-nitrobenzoic acid was read at 412 nm. The limit of detection of the test was 27 pg/ml. All the sample concentrations were determined by comparison with a recombinant standard curve in pg/mL.

Determination of Advanced Oxidation Protein Products (AOPP)

AOPP was determined in the plasma using the semiautomated method described by Witko-Sarsat et al. [23] AOPP results of oxidation of amino acid residues such as tyrosine, leading to the formation of dityrosine-containing protein cross-linking products detected by espectrophotometry.[19, 23] AOPP concentrations were expressed as micromoles per liter (umol/L) of chloramines-T equivalents.

Carbonyl protein contents

Carbonyl content was measured as an estimate of protein oxidative injury. Carbonyl proteins contents were determined as previously described by Panis et al. [24] Results are expressed in nmol ml-1 mg-1 total proteins.

Total radical-trapping antioxidant parameter (TRAP)

The TRAP was determined as reported by Reppeto et al. [25] This method detects hydrosoluble and/or liposoluble plasma antioxidants by measuring the chemiluminescence inhibition time induced by 2,2-azobis (2-amidinopropane). The system was calibrated with the vitamin E analog TROLOX, and the values of TRAP are expressed in equivalent of μM Trolox.

Determination of Sulfhydryl (SH) groups of proteins

Sulfhydryl groups of proteins were evaluated in plasma samples by a spectrophotometric assay based on 2,2-dithiobisnitrobenzoic acid (DTNB), as reported previously [26] and the results were expressed in μM.

Statistical analysis

Distribution of sex, race, and smoking was analyzed with Fisher's exact test or chi-square test. Comparisons between control group and SLE patients, and SLE group with insulin resistance or not were done using the Mann-Whitney test and data were expressed as the median (25-75 percentiles). Correlations were evaluated by Spearman's rank correlation. The results were considered significant when $p<0.05$. A statistical analysis program (Graph Pad Prism version 4.0) was used for evaluations.

RESULTS

Clinical characteristics of the patients with SLE are shown in table 1. There were no differences between the control group and SLE patients in relation to age, gender, ethnicity, BMI and ALT levels. However, patients with SLE presented higher AST ($p<0.0001$) and GGT levels ($p<0.001$). With regard to oxidative stress, patients with SLE showed higher serum lipid hydroperoxides ($p=0.0002$) and SH levels ($p<0.0001$) and lower TRAP corrected by UA levels ($p=0.0003$). Nevertheless, SLE patients had lower serum isoprostane ($p<0.0001$) and carbonyl protein levels ($p=0.05$) than controls.

Table 2 presents the clinical and laboratorial data of SLE patients with and without IR. There was no difference between the groups in relation to age, gender, ethnicity, smoking, duration of the disease, disease activity, SLEDAI score, positive anti-dsDNA, serum C4 levels, AST

and ALT. The frequency of patients who used prednisone, prednisone dose, current immunosuppressive drugs and antimalarials also showed no difference between the groups. However, SLE patients with IR had higher BMI ($p<0.0001$), serum C3 levels ($p=0.03$), GGT ($p=0.001$) and frequency of patients who used antihypertensive drugs ($p=0.001$) compared to SLE patients without IR. SLE patients with IR also presented a trend to use higher prednisone doses ($p=0.07$) compared to SLE patients without IR.

With regard to oxidative stress parameters, SLE patients with IR showed no differences in lipid hydroperoxides levels, but had higher AOPP ($p=0.03$), and lower SH ($p=0.0002$) and TRAP corrected by UA levels ($p=0.04$) when compared to SLE patients without IR (Table 3). However, SLE patients with IR presented lower serum isoprostane ($p=0.05$) and carbonyl protein levels ($p=0.04$) when compared to SLE patients without IR.

SLE patients showed significantly lower serum iron levels ($p<0.0001$) in relation to controls and SLE patients with IR presented lower serum iron levels ($p=0.04$) compared to SLE patients without IR (Figure 1). On the other hand, serum ferritin levels were significantly higher in SLE patients ($p=0.0006$) when compared to controls and SLE patients with IR presented higher serum ferritin levels ($p=0.01$) compared to SLE patients without IR (Figure 2).

Patients with SLE showed that insulin resistance was inversely correlated to TRAP/UA ($r=-0.2724$, $p=0.008$) and serum ferritin was positively correlated with AOPP ($r=0.2870$, $p=0.004$) (Figure 3).

DISCUSSION

The main findings of the present study are that iron metabolism state verified by increased ferritin is associated with both oxidative stress and insulin resistance in patients with SLE; the direct correlation verified between ferritin and AOPP and the inverse correlation between HOMA-IR and TRAP/UA reinforce these findings.

In general, our data showed that patients with SLE had increased oxidative stress and decreased antioxidant defenses when compared to healthy subjects. The increased oxidative stress was shown by hydroperoxides and AOPP and by decreased TRAP/UA ratio. Uric acid concentration is responsible for 60% of plasmatic total antioxidant capacity. Thus, a correction of total antioxidant capacity based on uric acid concentration is needed. [27] In

addition, GGT, a precocious and sensitive marker of oxidative stress [28], was also increased in patients with SLE, mainly in SLE patients with IR. Of note, the increase of serum GGT may predict the beginning of the MS and the incidence of cardiovascular disease [29] and in a previous study of our group GGT levels were positively correlated with HOMA-IR in patients with metabolic syndrome. [3] Moreover, GGT is also a non-specific marker of liver function. In a previous study, [19] we also reported that AST and GGT levels were enhanced in SLE patients, but only GGT levels correlated with protein oxidation. In addition, in the present study, GGT, but not AST, also increased in the group of SLE patients with IR. All together, these data seem to suggest GGT participation in generating and maintaining oxidative stress in patients with SLE.

SLE patients with IR had a higher BMI than SLE patients without IR. This result could interfere with oxidative stress measurements. However, in a previous study, we have shown that only overweight patients with IR had increased oxidative stress when compared to overweight patients without IR. [27] In addition, in some classical inflammatory diseases, like systemic lupus erythematosus, the higher MetS prevalence is better explained by insulin resistance rather than obesity [19, 30] reinforcing the importance of insulin sensitivity as it was proposed in the original concept given by Reaven. [31]

A protein complex with 24 peptide subunits (L and H forms) assembled into a hollow spherical shell forms ferritin**.** The expression of ferritin is under delicate control and is regulated at both the transcriptional and posttranscriptional levels by intracellular iron, inflammation, oxidative stress products, hormones, and growth factors, throughout heavy-chain (H) transcription and translation. [9] The H-ferritin is primarily responsible for the ferroxidase activity of the ferritin complex and this activity is essential for uptake of free iron, whereas the L-ferritin facilitates the storage of iron into the ferritin core. The efficient storage of iron requires the cooperation of both ferritin subunits. [32] Elevated serum ferritin levels in inflammatory states are due to release from damaged tissues, increased synthesis, and decreased clearance. The activation of inflammatory cytokines increases the transcription of ferritin messenger RNA in macrophage, which may subsequently transfer ferritin to hepatocytes. [33] Most of the stimuli related to inflammation and directed to ferritin synthesis seem to upregulate H-ferritin preferentially over the L form, thus determining an increase of catalytic sites and a reduction of cell iron availability. Therefore, ferritin in inflammatory tissue contains more iron with increased inflammation and some previous studies have demonstrated that serum ferritin is a useful marker of disease activity in patients with SLE. [34, 35]

The inflammatory process per se can cause oxidative stress and IR, but on the other side, oxidative stress and IR both can cause enhanced ferritin levels by different mechanism. Hyperinsulinemia may be directly responsible for the accumulation of iron in the liver, because insulin can stimulate cellular iron uptake by a mechanism of transferrin receptor externalization. In the meantime, increased oxidative stress may generate superoxide radicals, which can cause the release of ferrous iron from ferritin. [12, 13] Iron is a transitional metal that can convert poorly reactive free radicals into highly reactive moities, which can cause oxidative damage to cells and tissues. [16, 36] Unbound intracellular ferrous iron is capable of generation free radicals and ROS through Fenton chemistry causing lipid peroxidation, DNA breaks, and others forms of cellular damage. [15] Although the majority of reports and findings support the notion of the deleterious effects of ferritin, it has been shown that H-ferritin can sequester Fe (II) in the solution and reduce lipid peroxidation induced by Fenton-type reaction, and that iron-loaded ferritin can bind large amounts of nitric oxide molecules in different sites of the structure.(16) Therefore, in some circumstances it may play an important role in the protection against oxidative damage. As expected, SLE patients presented lower iron levels and higher ferritin levels than controls; however, these findings were also verified in SLE patients with RI when compared to SLE patients without RI. In addition, serum ferritin levels were directly correlated with protein oxidation, measured by AOPP. All together, these data allow hypothesize that ferritin seem to contribute, at least in part, to the redox imbalance verified in SLE patients.

In the present study, some traditional pro oxidant markers, such as isoprostanes and carbonyl proteins as well as SH, which reflects antioxidant defenses, showed unexpected behaviors. The decrease in the aforementioned pro oxidant markers and the increase in SH can be attributed to several causes and some issues must be evaluated to have an overall view. 1º) To evaluate the redox state of a determined pathological condition, it is necessary to use a robust and complete methodology approach. In this context, the interpretation of the several methods will allow a better understanding of the oxidative phenomena, which occurs. [19, 37]. 2º)Although oxidative stress has been reported to have deleterious effects, it also can have beneficial effects. Some pro oxidant decreased levels may be related to the disease pathophysiology and to the role of reactive substances and their metabolites in redox signaling. For instance, irreversible modifications induced by oxidative stress, such as protein carbonylation, are generally associated with permanent loss of protein function and may lead to the progressive accumulation of the damage proteins. In cells, the level of the progressive

accumulation of modified proteins reflects the balance between the rate of protein oxidation and the rate of oxidized protein degradation, and is dependent on multiple factors that influence the levels of pro-and antioxidants and the levels of proteases that catalyse the degradation of oxidized proteins residues. [38] The modulation of protein function by ROS may be in many ways analogous to phosphorylation, except that protein modification no longer occurs on specific serine, threonine, or tyrosine residues, but instead on redox-sensitive Cys residues. [39] Thus, conceivably different diseases in different phases may present different oxidative stress marker profiles. [40] 3º) All of the currently available methods for the detection of ROS are, by definition, indirect (because detector compounds are required) and semiquantitative (because specific ROS are in competition with other biomolecules that can react with these detector molecules). However, if the redox chemistries of the various detector molecules are carefully considered and the potential limitations of these assays are understood, they can provide useful information on the production and regulation of ROS in biological systems. [40] Thus, the hypothesis that the medications patients were receiving for SLE could have affected concentrations of carbonyl proteins and F2 isoprostanes [41] may not be underestimated. 4º) Protein cysteinyl thiols appear especially sensitive to ROS attack. One major means of protein redox regulation is mediated by the formation or reduction of disulphides, including mixed disulphides with low-molecular-mass thiols. [39] Our data suggest that increased sulfhydryl group (thiol), an indicator of antioxidant defense, in patients with SLE, can be attributed to concomitant homocysteine increase, a low-molecular-mass thiol.

In conclusion, this study showed that IR increases oxidative stress in patients with SLE and that increased ferritin whatever its cause, the inflammatory process per se or hyperinsulinemia, can favor the redox process. In addition, our data showed the importance of measuring oxidative stress with several methodologies with different assumptions. The understanding of the complex redox mechanisms involved in the context of the disease will proportionate a more accurate view of the phenomena.

Acknowledgments

This work was supported by the University of Londrina Research Funds (FAEPE).

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Figure legends

Figure 1: Serum iron levels in healthy subjects (controls) and in systemic lupus erythematosus (SLE) patients and serum iron levels in SLE patients with (HOMA≥2.11) or without (HOMA<2.11) insulin resistance. HOMA: homeostasis model assessment. Mann-Whitney test. Data are median (25-75%). *p < 0.0001 when compared to control group; **p= 0.04 when compared to HOMA <2.11.

Figure 2: Serum ferritin levels in healthy subjects (controls) and in systemic lupus erythematosus (SLE) patients and serum ferritin levels in SLE patients with (HOMA≥2.11) or without (HOMA<2.11) insulin resistance. HOMA: homeostasis model assessment Mann-Whitney test. Data are median (25-75%). *p =0.0006 when compared to control group; **p= 0.01 when compared to HOMA <2.11.

Figure 3: Spearman's correlation between: a. insulin resistance evaluated by Homeostasis Model Assessment – Insulin Resistance (HOMA-IR) and total antioxidant capacity evaluated by total radical-trapping antioxidant parameter **(**TRAP) (r=-0.2724, p= 0.008); b. ferritin and advanced oxidation protein products (AOPP) in patients with systemic lupus erythematosus (r=0.2870, p=0.004)

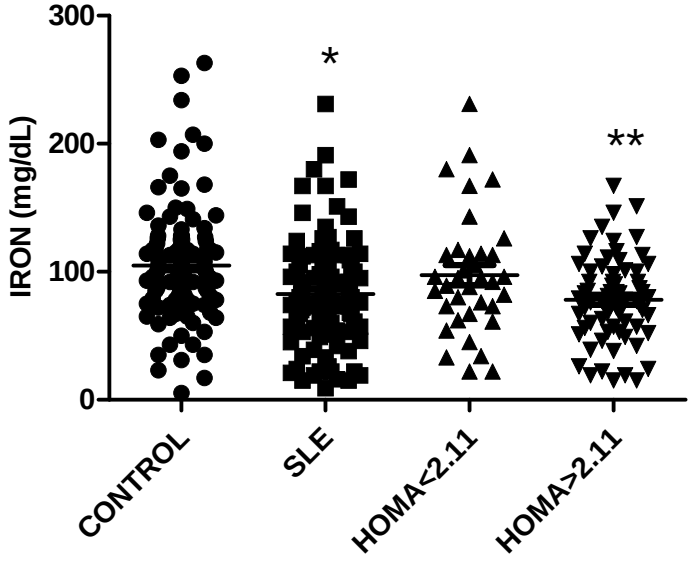

Figure 1

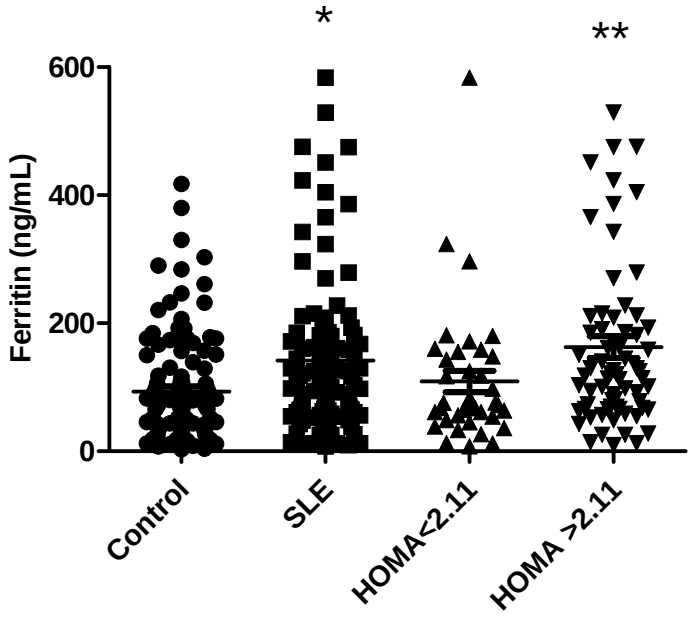

Figure 2:

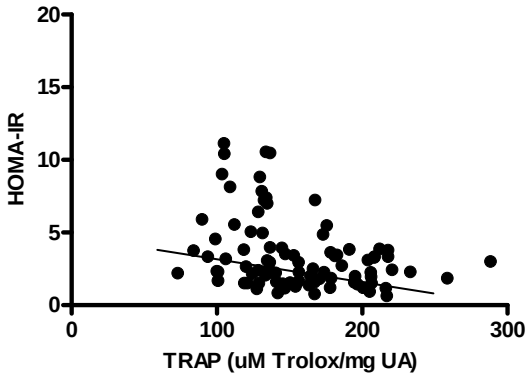

Figure a

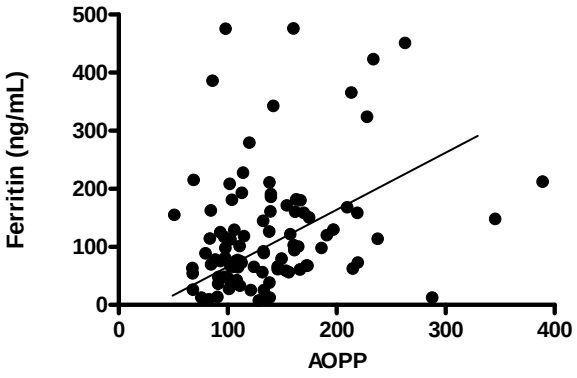

Figure b

Figure 3

Table 1: Clinical, biochemical and oxidative stress parameters of patients with systemic lupus erythematosus (SLE) and controls.

	Controls (n=125)	SLE (n=111)	P Value
Age (years)	40.0 (32.0-47.0)	43.0 (32.0-53.0)	0.126
Sexo (female/male)*	110/15	103/8	0.215
Smoking (Yes/No)*	11/114	7/104	0.624
BMI (kg/m^2)	26.2 (24.2-29.5)	27.6 (23.8-32.2)	0.313
AST	18.0 (15.0-23.0)	22.0 (19.0-27.3)	<0.0001
ALT	33.0 (29.0-40.0)	35.0 (29.0-41.5)	0.368
GGT	27.0 (20.0-39.0)	34.0 (24.0-52.0)	0.0014
Hidroperoxides (cpm)	14250 (11490-17970)	19040 (13130-28670)	0.0002
Isoprostanes (pg/mL)	231.5 (145.1-311.7)	30.35 (21.60-59.95)	<0.0001
AOPP (umol/L of chloramines-T equivalents)	136.3 (102.2-175.1)	132.5 (101.6-165.5)	0.503
Carbonyl proteins (nmol ml-1 mg-1 total proteins)	66.00 (54.76-83.89)	61.94 (38.84-81.08)	0.045
Sulfhydril proteins (μM)	0.306 (0.242-0.380)	0.371 (0.316-0.413)	<0.0001
Homocysteine (umol/L)	9.99 (8.47-11.81)	11.36 (9.60-14.31)	0.001
TRAP/UA (μM Trolox/mg UA)	180.3 (148.7-213.3)	150.4 (128.8-194.5)	0.0003

Mann-Whitney test. *Chi-square test. Data are median (25%– 75%). BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; AOPP, advanced oxidation protein products; TRAP, total radical-trapping antioxidant parameter, UA, uric acid

Table 2: Clinical and laboratory characteristics of patients with systemic lupus erythematosus (SLE) with (HOMA ≥2.11) and without (HOMA < 2.11) insulin resistance

	HOMA<2.11 (n=39)	HOMA ≥2.11 (n=72)	p
Age (years)	43.0 (32.0-55.0)	42.0 (31.8-51.3)	0.867
Gender (female/male)*	35/4	68/4	0.448
Caucasian (Y/N)*	2/37	5/67	0.707
Smoking (Y/N)*	2/37	5/67	0.7071
BMI (kg/m^2)	23.9 (21.9-25.9)	29.9 (26.2-33.5)	<0.0001
Disease Duration (years)*	8.5 (3.8-17.0)	10.0 (4.0-14.0)	0.805
Atividade Y/N**	16/23	30/42	0.786
Anti-dsDNA Positive (Y/N)**	7/32	14/58	0.848
SLEDAI Score	0 (0-2)	0 (0-2)	1.000
C3 (mg/dL)	105.0 (89.0-121.0)	116.0 (98.8-136.5)	0.030
C4 (mg/dL)	20.15 (13.75-24.50)	20.10 (13.75-25.90)	0.628
AST	22.0 (19.0-26.0)	22.0 (19.0-28.0)	0.898
ALT	36.0 (28.8-46.0)	35.0 (29.0-41)	0.275
GGT	27.0	39.0	0.001

	(19.0-42.0)	(28.0-63.5)	
Therapy			
Prednisone (Y/N)*	35/4	67/05	0.717
Prednisone (mg/day)	7.5 (5.0-20.0)	10.0 (7.5-20.0)	0.072
Antimalarials (Y/N)**	22/17	51/21	0.126
Current Immunosuppressive (Y/N)**	14/25	24/48	0.713
Antihypertensive drugs (Y/N)*	4/35	29/43	0.001

Mann-Whitney test. *Fisher´s exact test. **Chi-square test. Data are median (25%– 75%). BMI, body mass index; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HOMA-IR = Homeostasis Model of Assessment Insulin Resistance. Y, yes; N, no.

Table 3: Oxidative stress biomarkers, total antioxidant capacity and homocysteine of patients with systemic lupus erythematosus (SLE) with (HOMA ≥2.11) and without (HOMA < 2.11) insulin resistance

	SLE HOMA<2.11 (n=39)	HOMA ≥2.11 (n=72)	P
Hidroperoxides (cpm)	19040 (14780-27620)	19210 (12650-28040)	0.689
Isoprostanes (pg/mL)	38.30 (26.25-89.52)	27.30 (18.43-48.70)	0.050
AOPP (umol/L of chloramines-T equivalents)	109.4 (97.12-150.1)	138.4 (104.2-174.9)	0.030
Carbonyl proteins (nmol ml-1 mg-1 total proteins)	72.61 (43.63-99.51)	55.50 (36.30-70.23)	0.040
Sulfhydril proteins (μM)	0.447 (0.397-0.496)	0.360 (0.308-0.392)	0.0002
Homocysteine (umol/L)	10.93 (9.36-13.62)	11.51 (9.67-14.31)	0.645
TRAP (μM Trolox/mg UA)	167.2 (141.9-200.6)	136.5 (121.7-182.0)	0.040

Mann-Whitney test. Data are median (25%– 75). AOPP, advanced oxidation protein products; TRAP, total radical-trapping antioxidant parameter ;UA, uric acid; HOMA-IR = Homeostasis Model of Assessment Insulin Resistance.

5 CONCLUSÃO

Os resultados obtidos neste trabalho nos permitem concluir que:

1 Pacientes com LES apresentam maior chance de desenvolverem RI e SM e esta aumenta com a atividade da doença. O aumento do IMC e a corticoterapia parecem ser os principais fatores envolvidos.

2 Pacientes com LES apresentam aumento na freqüência de hipertensão arterial quando comparados indivíduos controles, pareados pelo gênero, etnia, idade e IMC.

3 A atividade da doença aumenta a probabilidade de desenvolver hipertensão arterial em pacientes com LES.

4 Pacientes com LES apresentam aumento de estresse oxidativo lipídico e protéico, aumento dos níveis séricos de metabólitos de óxido nítrico e diminuição da capacidade antioxidante total quando comparados à indivíduos controles.

5 Pacientes com LES apresentam aumento da resposta imune Th1, Th2 (exceto IL-4) e Th17 quando comparados a indivíduos controles.

6 A atividade da doença aumenta a relação pró-inflamatória/Th2 (IL-12/IL-4) e o estresse oxidativo e ambos estão relacionados à maior frequência de hipertensão arterial nesta situação.

7 Pacientes com LES e RI apresentam aumento de estresse oxidativo, dos níveis séricos de ferritina e diminuição das defesas antioxidantes e ferro sérico

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ANEXOS

ANEXO A

Escore SLEDAI

SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE ACTIVITY INDEX
SELENA MODIFICATION

Physicians Global Assessment _____

0 None 1 Mild 2 Med 3 Severe

SLEDAI SCORE

Check box: If descriptor is present at the time of visit or in the proceeding 10 days

Wt	Present	Descriptor	Definition
8	☐	Seizure	Recent onset. Exclude metabolic, infectious or drug cause
8	☐	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucinations, incoherence, marked loose associations, impoverished thought content, marked illogical thinking, bizarre, disorganized, or catatonic behavior. Excluded uremia and drug causes.
8	☐	Organic Brain Syndrome	Altered mental function with impaired orientation, memory or other intelligent function, with rapid onset fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious or drug causes.
8	☐	Visual Disturbance	Retinal changes of SLE. Include cytoid bodies, retinal hemorrhages, serious exodate or hemorrhages in the choroids, or optic neuritis. Exclude hypertension, infection, or drug causes.
8	☐	Cranial Nerve Disorder	New onset of sensory or motor neuropathy involving cranial nerves.
8	☐	Lupus Headache	Severe persistent headache: may be migrainous, but must be non-responsive to narcotic analgesia.
8	☐	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis
8	☐	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual, infarction, splinter hemorrhages, or biopsy or angiogram proof of vasculitis
4	☐	Arthritis	More than 2 joints with pain and signs of inflammation (i.e. tenderness, swelling, or effusion).
4	☐	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/adolase or electromyogram changes or a biopsy showing myositis.
4	☐	Urinary Casts	Heme-granular or red blood cell casts
4	☐	Hematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause.
4	☐	Proteinuria	>0.5 gm/24 hours. New onset or recent increase of more than 0.5 gm/24 hours.
4	☐	Pyuria	>5 white blood cells/high power field. Exclude infection.
2	☐	New Rash	New onset or recurrence of inflammatory type rash.
2	☐	Alopecia	New onset or recurrence of abnormal, patchy or diffuse loss of hair.
2	☐	Mucosal Ulcers	New onset or recurrence of oral or nasal ulcerations

2	□	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening.
2	□	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram confirmation.
2	□	Low Complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory.
2	□	Increased DNA binding	>25% binding by Farr assay or above normal range for testing laboratory.
1	□	Fever	>38°C. Exclude infectious cause
1	□	Thrombocytopenia	<100,000 platelets/mm3
1	□	Leukopenia	<3,000 White blood cell/mm3. Exclude drug causes.

_____ TOTAL SCORE (Sum of weights next to descriptors marked present)

Mild or Moderate Flare □	**Severe Flare** □
□ Change in SLEDAI > 3 points	□ Change in SLEDAI > 12
□ New/worse discoid, photosensitive, profundus, cutaneous vasculitis, bullous lupus Nasopharyngeal ulcers Pleuritis Pericarditis Arthritis Fever (SLE)	□ New/worse CNS-SLE Vasculius Nephritis Myositis Pk < 60.000 Home anemia: Hb <7% or decrease in Hb > 3% **Requiring:** double prednisone Prednisone>0.5 mg/kg/day hospitalization
□ Increase in Prednisone, but not to >0.5 mg/kg/day	□ Prednisone >0.5 mg/kg/day
□ Added NSAID or Plaquenil	□ New Cytoxan, Azathioprine, Methotrexate, Hospitalization (SLE)
□ ≥1.0 Increase in PGA, but not to more than 2.5	□ Increase in PGA to > 2.5

ANEXO B

Instruções para Autores nos periódico nos quais os artigos foram submetidos

Scandinavian Journal of Rheumatology

2010	**Impact**		**Factor:**		**2.594**
5-year	**Impact**		**Factor:**		**2.377**
Ranking:		**15/29**			**(Rheumatology)**
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Instructions for Authors

Web-based Sub

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The Scandinavian Journal of Rheumatology all new manuscripts must be submitted via the web-based manuscript submission and handling system ManuscriptCentral: **http://mc.manuscriptcentral.com/scandjrheumatol**

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The Editorial Office will help you with any enquiries about the journal's requirements.

General information

The Scandinavian Journal of Rheumatology is the official journal of the Scandinavian Society for Rheumatology. The Journal publishes original research on all aspects of clinical and experimental rheumatology. Original reports, review articles, informative case reports, and letters are equally welcome.

Author and Disclosure Statement

Submission of a manuscript to Scandinavian Journal of Rheumatology is understood to imply that it has not previously been published (except in abstract form) and is not being considered for publication elsewhere.

Manuscripts submitted under multiple authorship are reviewed under the assumption that: 1) all authors listed concur with the submitted version of the manuscript and with the listing of the authors; 2) authorship credit is based on important contributions in one or more of the following areas: conception and design, analysis and interpretation of data, drafting of the manuscript or making intellectual contribution to its content; 3) the final manuscript has been explicitly approved by the responsible authorities in the laboratory or institution were the work was carried out.

Manuscript preparation

Submission items include:

A cover letter

A title page (the Scandinavian Journal of Rheumatology uses a double-blinded review process and therefore, you will be asked to save the title page as a separate file for upload)

The manuscript should include the abstract (if required by article type), main text, references, and figure legends

The manuscript should be formatted with a font size no smaller than 12 point. Times, Times Roman, Courier, Helvetica and Arial are the recommended fonts for the best quality conversion, and with a 2 cm margin at both sides of the text and 3 cm wide margins at the top and bottom of the page.

Special characters and symbols, use the Symbol font.

All abbreviations (click to open pdf with list) must be defined.

Files should be labeled with appropriate and descriptive file names (e.g., SmithText.doc, Fig1.tif, Table3.doc).

Do not upload your text as a PDF.

Authors are encouraged to suggest names of 3-4 persons who might be considered suitable reviewers of their work.

Title page

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Articles

Articles represent a substantial body of clinical or laboratory work. The paper should be presented in sections as listed below.

Length of manuscript: Abstract: 250 words; Introduction: 500 words, Discussion: 1500; Methods and Results: no limit. If the manuscript exceeds this word count, authors will be

required to revise the paper. Permission to exceed these guidelines must be obtained from the Editorial Office.

Abstract

Provide a structured abstract of not more than 150–250 words, using the headings Objectives, Methods, Results and Conclusions.

Key words (maximum of 10)

Use up to 10 key words that reflect the content of the article and will facilitate it being found through Internet searches. Key words should be given beneath the abstract in the box provided in the online submission process.

Introduction

Provide a brief description of the background that led to the study.

Material and methods

Identify details relevant to the conduct of the study. Statistical methods should be clearly explained at the end of this section.

Subheadings that add clarity of presentation are encouraged.

Results

Work should be reported in SI units. Undue repetition in text and tables should be avoided. Comment on validity and significance of results is appropriate but broader discussion of their implication is restricted to the next section.

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When reporting experiments on human subjects, a statement is required that the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 1983. Do not use patients' names, initials, or hospital numbers, especially in illustrative material. Papers including animal experiments or clinical trials must be accompanied by an approval by the local ethics committee and, in the case of animal experiments of any relevant local Licensing Authority. Please give date of issue and registration number in a covering letter. Identifying information should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published.

Authors reporting on experimental work on humans should, where relevant, submit evidence that the work has been approved by an institutional clinical research panel or its equivalent.

Discussion

Place the nature and findings of the study in the context of other relevant published data. Avoid undue extrapolation from the study topic.

Acknowledgments

Individuals who have made substantial contributions to the study but who are not included in authorship may be acknowledged. The source of support in the form of grants, equipment and drugs of those involved must be stated.

References

In the text references are given as numbers in brackets on the line in order of appearance. At the end of the paper references are given in accordance with the Vancouver system, cited by the numerical system, and listed in the order cited in the text. *List all authors when six or less; when seven or more, list only the first six and add et al*. Journal titles are abbreviated in accordance with the style in Index Medicus.

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Books 3. Maddison PJ, Isenberg DA, Woo P, Glass DN, eds. Oxford textbook of rheumatology, Vol. 1. Oxford, New York, Tokyo: Oxford University Press, 1993. 4. Choy EHS, Panayi GS. Therapeutics of the future. In: JJF Belch. RB Zurier, eds. Connective tissue diseases. London: Chapman & Hall. 1995:355-77.

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Images should be of sufficiently high quality with respect to detail, contrast and fineness of grain to withstand the inevitable loss of contrast and detail inherent in the printing process.

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Short reports organized as follows: brief abstract, case report including treatment, differential diagnosis, and comment and to be restricted to no more than 1500 words, 3 figures or tables, and up to 15 references.

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Original observations relating to short clinical or laboratory studies or case reports may be presented as a Letter to the Editor. Letters are not divided into sections and are limited to no more than 700 words and 10 references; one table and one figure may be included. Instructions for references, tables, and figures are the same as for full length articles.

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JOURNAL OF TRANSLATIONAL MEDICINE

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General guidelines of the journal's style and language are given below.

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Manuscripts for Research Articles submitted to *Journal of Translational Medicine* should be divided into the following sections (in this order):

- Title page
- Abstract
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- Keywords
- Background
- Methods
- Results and discussion
- Conclusions
- List of abbreviations used (if any)
- Competing interests
- Authors' contributions
- Authors' information
- Acknowledgements
- Endnotes
- References
- Illustrations and figures (if any)
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The **Accession Numbers** of any nucleic acid sequences, protein sequences or atomic coordinates cited in the manuscript should be provided, in square brackets and include the corresponding database name; for example, [EMBL:AB026295, EMBL:AC137000, DDBJ:AE000812, GenBank:U49845, PDB:1BFM, Swiss-Prot:Q96KQ7, PIR:S66116].

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For reporting standards please see the information in the About section.

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The title page should:

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- list the full names, institutional addresses and email addresses for all authors
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Please note:

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- abbreviations within the title should be avoided

Abstract

The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: **Background**, the context and purpose of the study; **Methods**, how the study was performed and statistical tests used; **Results**, the main findings; **Conclusions**, brief summary and potential implications. Please minimize the use of abbreviations and do not cite references in the abstract. **Trial registration**, if your research reports the results of a controlled health care intervention, please list your trial registry, along with the unique identifying number (e.g. **Trial registration**: Current Controlled Trials ISRCTN73824458). Please note that there should be no space between the letters and numbers of your trial registration number. We recommend manuscripts that report randomized controlled trials follow the CONSORT extension for abstracts.

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Keywords

Three to ten keywords representing the main content of the article.

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Methods

The methods section should include the design of the study, the setting, the type of participants or materials involved, a clear description of all interventions and comparisons, and the type of analysis used, including a power calculation if appropriate. Generic drug names should generally be used. When proprietary brands are used in research, include the brand names in parentheses in the Methods section.

For studies involving human participants a statement detailing ethical approval and consent should be included in the methods section. For further details of the journal's editorial policies and ethical guidelines see 'About this journal'.

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Results and discussion

The Results and discussion may be combined into a single section or presented separately. Results of statistical analysis should include, where appropriate, relative and absolute risks or risk reductions, and confidence intervals. The Results and discussion sections may also be broken into subsections with short, informative headings.

Conclusions

This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

List of abbreviations

If abbreviations are used in the text they should be defined in the text at first use, and a list of abbreviations can be provided, which should precede the competing interests and authors' contributions.

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A competing interest exists when your interpretation of data or presentation of information may be influenced by your personal or financial relationship with other people or organizations. Authors must disclose any financial competing interests; they should also reveal any non-financial competing interests that may cause them embarrassment were they to become public after the publication of the manuscript.

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We suggest the following kind of format (please use initials to refer to each author's contribution): AB carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. JY carried out the immunoassays. MT participated in the sequence alignment. ES participated in the design of the study and performed the statistical analysis. FG conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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You may choose to use this section to include any relevant information about the author(s) that may aid the reader's interpretation of the article, and understand the standpoint of the author(s). This may include details about the authors' qualifications, current positions they hold at institutions or societies, or any other relevant background information. Please refer to authors using their initials. Note this section should not be used to describe any competing interests.

Acknowledgements

Please acknowledge anyone who contributed towards the article by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. Please also include the source(s) of funding for each author, and for the manuscript preparation. Authors must describe the role of the funding body, if any, in design, in the collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. Please also acknowledge anyone who contributed materials essential for the study. If a language editor has made significant revision of the manuscript, we recommend that you acknowledge the editor by name, where possible.

The role of a scientific (medical) writer must be included in the acknowledgements section, including their source(s) of funding. We suggest wording such as 'We thank Jane Doe who provided medical writing services on behalf of XYZ Pharmaceuticals Ltd.'

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References

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Koonin EV, Altschul SF, Bork P: **BRCA1 protein products: functional motifs.** *Nat Genet* 1996, **13:**266-267.

Article within a journal supplement
Orengo CA, Bray JE, Hubbard T, LoConte L, Sillitoe I: **Analysis and assessment of ab initio three-dimensional prediction, secondary structure, and contacts prediction.** *Proteins* 1999, **43**(Suppl 3):149-170.

In press article
Kharitonov SA, Barnes PJ: **Clinical aspects of exhaled nitric oxide.** *Eur Respir J*, in press.

Published abstract
Zvaifler NJ, Burger JA, Marinova-Mutafchieva L, Taylor P, Maini RN: **Mesenchymal cells, stromal derived factor-1 and rheumatoid arthritis [abstract].** *Arthritis Rheum* 1999, **42:**s250.

Article within conference proceedings
Jones X: **Zeolites and synthetic mechanisms.** In *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Edited by Smith Y. Stoneham: Butterworth-Heinemann; 1996:16-27.

Book chapter, or article within a book
Schnepf E: **From prey via endosymbiont to plastids: comparative studies in dinoflagellates.** In *Origins of Plastids. Volume 2.* 2nd edition. Edited by Lewin RA. New York: Chapman and Hall; 1993:53-76.

Whole issue of journal
Ponder B, Johnston S, Chodosh L (Eds): **Innovative oncology.** In *Breast Cancer Res* 1998, **10:**1-72.

Whole conference proceedings
Smith Y (Ed): *Proceedings of the First National Conference on Porous Sieves: 27-30 June 1996; Baltimore.* Stoneham: Butterworth-Heinemann; 1996.

Complete book
Margulis L: *Origin of Eukaryotic Cells.* New Haven: Yale University Press; 1970.

Monograph or book in a series
Hunninghake GW, Gadek JE: **The alveolar macrophage.** In *Cultured Human Cells and Tissues.* Edited by Harris TJR. New York: Academic Press; 1995:54-56. [Stoner G (Series Editor): *Methods and Perspectives in Cell Biology,* vol 1.]

Book with institutional author
Advisory Committee on Genetic Modification: *Annual Report.* London; 1999.

PhD thesis
Kohavi R: **Wrappers for performance enhancement and oblivious decision graphs.** *PhD thesis.* Stanford University, Computer Science Department; 1995.

Link / URL
The Mouse Tumor Biology Database [http://tumor.informatics.jax.org/mtbwi/index.do]

Link / URL with author(s)
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Dataset with persistent identifier
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Preparing additional files

Although *Journal of Translational Medicine* does not restrict the length and quantity of data included in an article, there may still be occasions where an author wishes to provide data sets, tables, movie files, or other information as additional files. Results that would otherwise be indicated as "data not shown" can and should be included as additional files. Since many weblinks and URLs rapidly become broken, *Journal of Translational Medicine* requires that all supplementary data are included as additional files rather than as a link to your own website. These files can be uploaded using the 'Additional Material files' button in the manuscript submission tool.

The maximum file size for additional files is 20 MB each, and files will be virus-scanned on submission.

Additional files will be linked to the final published article in the form supplied by the author, but will not be displayed within the article. They will be made available in exactly the same form as originally provided by the authors.

If additional material is provided, please list the following information in a separate section of the manuscript text, immediately following the tables (if any):

- File name (e.g. Additional file 1)
- File format including the three-letter file extension (including name and a URL of an appropriate viewer if format is unusual)
- Title of data
- Description of data

Additional files should be named "Additional file 1" and so on and should be referenced explicitly by file name within the body of the article, e.g. 'An additional movie file shows this in more detail [see Additional file 1]'.

Additional file formats

Ideally, file formats for additional files should not be platform-specific, and should be viewable using free or widely available tools. The following are examples of suitable formats.

- Additional documentation
    - PDF (Adode Acrobat)
- Animations
    - SWF (Shockwave Flash)
- Movies
    - MOV (QuickTime)
    - MPG (MPEG)
- Tabular data

- XLS, XLSX (Excel Spreadsheet)
- CSV (Comma separated values)

As with figure files, files should be given the standard file extensions. This is especially important for Macintosh users, since the Mac OS does not enforce the use of standard extensions. Please also make sure that each additional file is a single table, figure or movie (please do not upload linked worksheets or PDF files larger than one sheet).

Mini-websites

Small self-contained websites can be submitted as additional files, in such a way that they will be browsable from within the full text HTML version of the article. In order to do this, please follow these instructions:

1. Create a folder containing a starting file called index.html (or index.htm) in the root.
2. Put all files necessary for viewing the mini-website within the folder, or sub-folders.
3. Ensure that all links are relative (ie "images/picture.jpg" rather than "/images/picture.jpg" or "http://yourdomain.net/images/picture.jpg" or "C:\Documents and Settings\username\My Documents\mini-website\images\picture.jpg") and no link is longer than 255 characters.
4. Access the index.html file and browse around the mini-website, to ensure that the most commonly used browsers (Internet Explorer and Firefox) are able to view all parts of the mini-website without problems, it is ideal to check this on a different machine.
5. Compress the folder into a ZIP, check the file size is under 20 MB, ensure that index.html is in the root of the ZIP, and that the file has .zip extension, then submit as an additional file with your article.

Style and language

General

Currently, *Journal of Translational Medicine* can only accept manuscripts written in English. Spelling should be US English or British English, but not a mixture.

There is no explicit limit on the length of articles submitted, but authors are encouraged to be concise. There is also no restriction on the number of figures, tables or additional files that can be included with each article online. Figures and tables should be numbered in the order in which they are referred to in the text. Authors should include all relevant supporting data with each article.

Journal of Translational Medicine will not edit submitted manuscripts for style or language; reviewers may advise rejection of a manuscript if it is compromised by grammatical errors. Authors are advised to write clearly and simply, and to have their article checked by colleagues before submission. In-house copyediting will be minimal. Non-native speakers of English may choose to make use of a copyediting service.

Help and advice on scientific writing

The abstract is one of the most important parts of a manuscript. For guidance, please visit our page on Writing titles and abstracts for scientific articles.

Tim Albert has produced for BioMed Central a list of tips for writing a scientific manuscript. American Scientist also provides a list of resources for science writing.

Abbreviations

Abbreviations should be used as sparingly as possible. They should be defined when first used and a list of abbreviations can be provided following the main manuscript text.

Typography

- Please use double line spacing.
- Type the text unjustified, without hyphenating words at line breaks.
- Use hard returns only to end headings and paragraphs, not to rearrange lines.
- Capitalize only the first word, and proper nouns, in the title.
- All pages should be numbered.
- Use the *Journal of Translational Medicine* reference format.
- Footnotes are not allowed, but endnotes are permitted.
- Please do not format the text in multiple columns.
- Greek and other special characters may be included. If you are unable to reproduce a particular special character, please type out the name of the symbol in full. **Please ensure that all special characters used are embedded in the text, otherwise they will be lost during conversion to PDF.**

Units

SI units should be used throughout (liter and molar are permitted, however).

Manuscript Submission Guidelines

Lupus

9. Manuscript style

Authors are asked to write their manuscripts in English. Spelling and phraseology should conform either to standard UK English or to standard American English and should be consistent throughout the paper.

The Summary should not exceed 200 words. It should be written in a style that conveys the essential message of the paper in abbreviated form.

The Introduction should assume that the reader is knowledgeable in the field and should therefore be as brief as possible.

In the Materials and methods section, methods that have been published in detail elsewhere should not be described in detail. SI units should be used throughout the text.

9.1 File types
Only electronic files conforming to the journal's guidelines will be accepted. Preferred formats for the text and tables of your manuscript are Word DOC, and tiff or jpeg for figures (ideally figures will use journal colours).Â Please also refer to additional guideline on submitting artwork [and supplemental files] below.

9.2 Journal Style
Lupus conforms to the SAGE house style.Â Click here to review guidelines on SAGE UK House Style.

9.3 Reference Style
Lupus adheres to the SAGE Vancouver reference style. Click here to review the guidelines on SAGE Vancouver to ensure your manuscript conforms to this reference style.

It is important that references comply with the style of the journal. Exhaustive lists should be avoided. References should follow the Vancouver format, listed (double-spaced) in numerical order corresponding to the order of citation in the text.

All authors should be quoted for papers with up to six authors; for papers with more than six authors, the first three only should be quoted followed by et al.

No issue numbers should be quoted.
Abbreviations for titles of medical periodicals should conform to those used in the latest editions of Index Medicus and Current Contents. The first and last page numbers for each reference should be provided. Abstracts and letters must be identified as such.

Papers in press and papers already submitted for publication may be included in the list of references. No citation is required for work that is not yet submitted for publication.

Personal communications may be allocated a number and included in the list of references in the usual way or simply referred to in the text. Authors must obtain permission from the individual concerned to quote his or her unpublished work.

Examples of References:
Journal article:
1 Derksen RHWM, Bouma BN, Kater L. The association between the lupus anticoagulant and cerebral infarction in systemic lupus erythematosus. Scand J Rheumatol 1986; 15: 179-184.
Journal article, in press:
2 Mendonca LLF, Khamashta MA, Nelson-Piercy A, Hunt BJ, Hughes GRV. Nonsteroidal anti-inflammatory drugs as a possible cause for reversible infertility. Rheumatology (in press).
Journal article submitted for publication:
3 Khamashta MA, Cervera R, Asherson RA. Association of antibodies against phospholipids with heart valve disease in systemic lupus erythematosus (submitted for publication).

Complete book:
4 Wallace DJ, Dubois EL. Dubois' Lupus Erythematosus. Lea & Febiger, 1987, p 51.
Chapter in book:
5 Christian CL. Etiologic hypotheses for systemic lupus erythematosus. In: Lahita RG (ed) Systemic Lupus Erythematosus. Wiley, 1987, 65-79.
Abstract:
6 Valesini G, Luan FL, Falco M. Clonal analysis of affinity purified anticardiolipin antibodies. Clin Exp Rheumatol 1988; 6: 214 (abstract 102).
Letter to the Editor:
7 Sills EM. Systemic lupus erythematosus in a patient diagnosed as having Shulman disease. Arthritis Rheum 1988; 31: 694 (letter).

9.4. Manuscript Preparation
The text should be double-spaced throughout and with a minimum of 3cm for left and right hand margins and 5cm at head and foot. Text should be standard 10 or 12 point. SI units should be used throughout the text.

9.4.1 Keywords and Abstracts
The title, keywords and abstract are key to ensuring that readers find your article online through online search engines such as Google. Please refer to the information and guidance on how best to title your article, write your abstract and select your keywords by visiting SAGEâ€™s Journal Author Gateway Guidelines on How to Help Readers Find Your Article Online.

9.4.2 Corresponding Author Contact details
Provide full contact details for the corresponding author including email, mailing address and telephone numbers. Academic affiliations are required for all co-authors.

9.4.3 Guidelines for submitting artwork, figures and other graphics
For guidance on the preparation of illustrations, pictures and graphs in electronic format, please visit SAGEâ€™s Manuscript Submission Guidelines.

TABLES
Each table should be numbered consecutively with an Arabic numeral. Each should have a separate caption or title. Methods not described in the text and abbreviations should be explained at the foot of the table. Footnotes should be designated by superior lower case letters (a, b, c etc). Vertical lines should not be inserted in the table. Tables must be referred to specifically in the text of the paper.

FIGURES
Lettering should be planned for 50% reduction; text should be readable after reduction. Figures should be referred to as Figure 1, Figure 2 etc. Figures must be referred to specifically in the text of the paper.

Images should be supplied as bitmap based files (i.e. with .tiff or .jpeg extension) with a resolution of at least **300 dpi** (dots per inch). Line art should be supplied as vector-based, separate .eps files (not as .tiff files, and not only inserted in the Word or pdf file), with a resolution of **600 dpi**. Images should be clear, in focus, free of pixilation and not too light or dark.

Colour photographs and Figures - Important information:
Colour photographs and Figures, when accepted, will be published online. In the printed version, they will be in black and white (unless colour prints are paid for). Authors who submit in colour must ensure that their figures are of the highest definition for the black and white version otherwise these may not be accepted. In particular immuno-fluorescent and histological figures, as well as skin rashes, must be paid for in colour or omitted from the manuscript and replaced in a descriptive format. If, together with your accepted article, you submit usable colour figures, these figures will appear in colour online regardless of whether or not these illustrations are reproduced in colour in the printed version. For specifically requested colour reproduction in print, you will receive information regarding the possible costs from SAGE after receipt of your accepted article.

9.4.4 Guidelines for submitting supplemental files
The journal may be able to host approved supplemental materials online, alongside the full-text of articles. Supplemental files will be subjected to peer-review alongside the article. Please contact the Editor (editorial@lupusjournal.co.uk) in the first instance.Â For more information please refer to SAGEâ€™s Guidelines for Authors on Supplemental Files.

9.4.5 English Language Editing
Non-English speaking authors who would like to refine their use of language in their manuscripts should have their manuscript reviewed by colleagues with experience of preparing manuscripts in English.

Alternatively it might be useful to consider using a professional editing service. Visit http://www.uk.sagepub.com/journalgateway/msg.htm for further information.

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10. After acceptance

10.1 Proofs
We will email a PDF of the proofs to the corresponding author. Corrections should be limited to typographical amendments. Authors' approval will be assumed if corrections are not returned by the date indicated.

10.2 E-Prints and Complimentary Copies
SAGE provides authors with access to a PDF of their final article. For further information please visit Offprints and Reprints . We additionally provide the corresponding author with a complimentary copy of the print issue in which the article appears up to a maximum of 5 copies for onward supply by the corresponding author to co-authors.

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11. Further information

If you would like to discuss your paper prior to submission, please contact the Editor:

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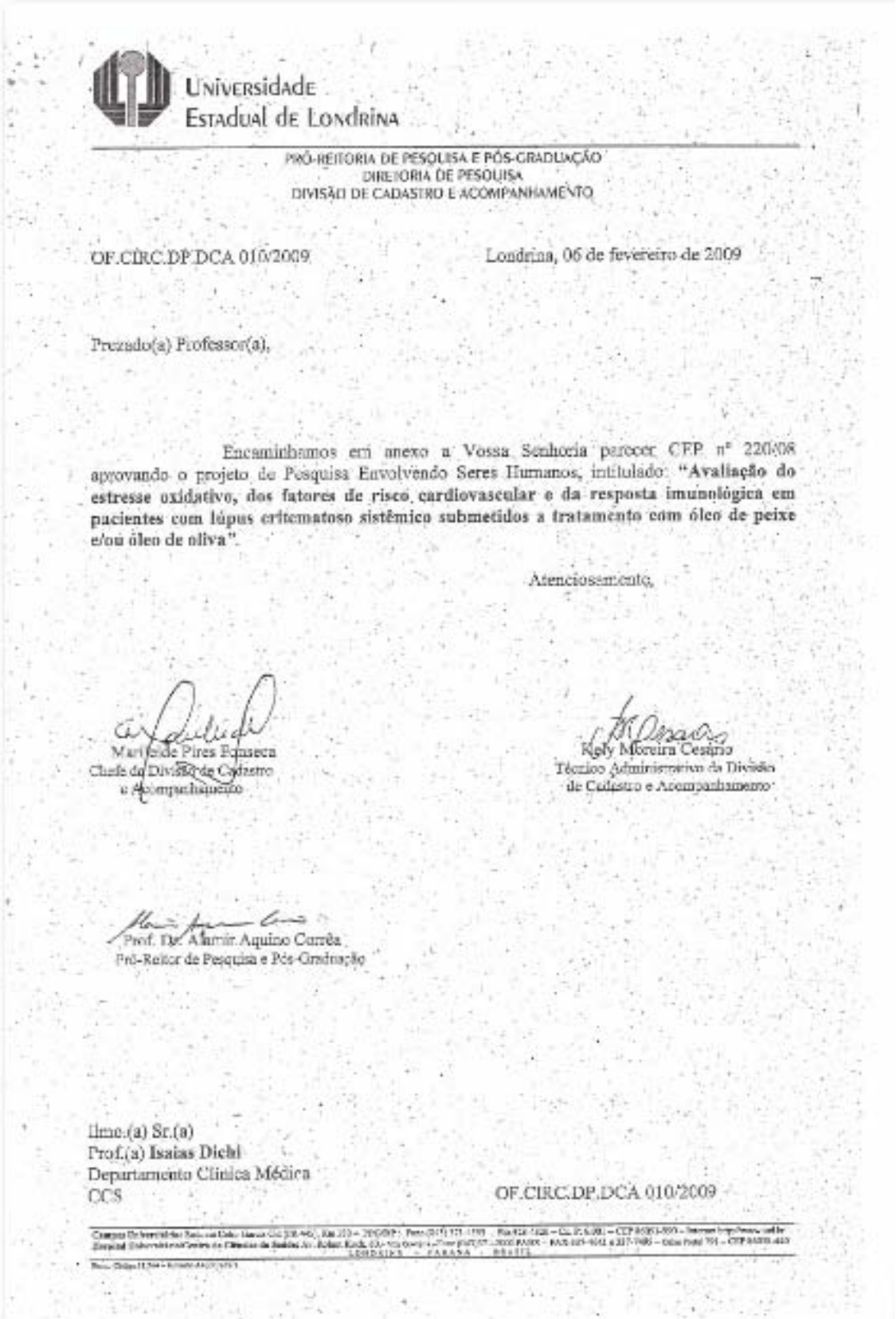
APÊNDICES

APÊNDICE A

Protocolo de Aprovação no Comitê de Ética



Universidade Estadual de Londrina

PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
DIRETORIA DE PESQUISA
DIVISÃO DE CADASTRO E ACOMPANHAMENTO

OF.CIRC.DP.DCA 010/2009 Londrina, 06 de fevereiro de 2009

Prezado(a) Professor(a),

Encaminhamos em anexo a Vossa Senhoria parecer CEP nº 220/08 aprovando o projeto de Pesquisa Envolvendo Seres Humanos, intitulado: **"Avaliação do estresse oxidativo, dos fatores de risco cardiovascular e da resposta imunológica em pacientes com lúpus eritematoso sistêmico submetidos a tratamento com óleo de peixe e/ou óleo de oliva".**

Atenciosamente,

Marli de Pires Fonseca
Chefe da Divisão de Cadastro e Acompanhamento

Kely Moreira Cesário
Técnico Administrativo da Divisão de Cadastro e Acompanhamento

Prof. Dr. Almir Aquino Corrêa
Pró-Reitor de Pesquisa e Pós-Graduação

Ilmo.(a) Sr.(a)
Prof.(a) **Isaias Dichi**
Departamento Clínica Médica
CCS

OF.CIRC.DP.DCA 010/2009

Universidade Estadual de Londrina

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná
Registro CONEP 268

PARECER CEP Nº 220/08 CAAE Nº 0215.0.268-000-08	Londrina, 16 de dezembro de 2008.

PESQUISADOR(A): ISAIAS DICHI

Ilmo(a) Sr(a)

O "Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná" de acordo com as orientações da Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares, APROVA a execução do projeto:

"AVALIAÇÃO DOS ESTRESSE OXIDATIVO, DOS FATORES DE RISCO CARDIOVASCULAR E DA RESPOSTA IMUNOLÓGICA EM PACIENTES COM LÚPUS ERITEMATOSO SISTÊMICO SUBMETIDOS A TRATAMENTO COM ÓLEO DE PEIXE E/OU ÓLEO DE OLIVA".

Informamos que a Sr(a) deverá comunicar, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá apresentar ao CEP/UEL relatório final da pesquisa.

Situação do Projeto: **APROVADO**

Atenciosamente,

Profª. Dra. Ester M. D. Dalla Costa
Comitê de Ética em Pesquisa - CEP/UEL
Coordenadora

APÊNDICE B

Termo de Consentimento Livre e Esclarecido

Projeto de Pesquisa: **Avaliação do estresse oxidativo, dos fatores de risco cardiovascular e da resposta imunológica em pacientes com Lúpus Eritematoso Sistêmico submetidos a tratamento com óleo de peixe e/ou óleo de oliva.**

Você, _____, idade _____ anos, residente em _____, na cidade de _____, telefone _____, recebeu todas as informações necessárias a respeito da realização deste projeto de pesquisa e declara estar completamente esclarecido sobre a forma como a pesquisa será realizada, não tendo dúvidas sobre a natureza e os procedimentos aos quais será submetido. Serão necessárias 2 coletas de sangue, uma no início do estudo e uma no término. As informações e os resultados obtidos nesta pesquisa não serão utilizados para outros fins e serão mantidos sob sigilo médico. Para tanto, assine aqui o consentimento para ser incluído no estudo, assumindo o compromisso de permitir a coleta de sangue venoso para a realização dos exames laboratoriais necessários. Declara também que está ciente de que sua participação é voluntária, de que será informado dos resultados dos exames realizados, de que não terá nenhum ônus e de que poderá deixar de participar do estudo a qualquer momento se assim o desejar, sem restrições de qualquer espécie e sem prejuízo ao seu atendimento.

Londrina, _____

Assinatura do participante ou responsável: _____

Assinatura do responsável pela pesquisa: _____