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ORIGINAL RESEARCH ARTICLE

Association of Oxidative Stress with Disease Activity and Damage in Systemic Lupus Erythematosus: A Cross Sectional Study from a Tertiary Care Centre in Southern India

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Abstract To study oxidative stress in systemic lupus erythematosus (SLE) by estimating serum oxidised LDL (OxLDL), 8-hydroxy-2′-deoxyguanosine (8-OHdG), malondialdehyde (MDA), and total anti-oxidant status and to correlate with SLE disease activity and disease damage. Eighty SLE patients satisfying the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) 2012 criteria and 80 healthy controls were studied. Exclusion criteria were infections, renal insufficiency, other connective tissue diseases, drug-induced lupus, smoking, alcohol consumption. Disease activity was measured by SLE disease activity index-2 K (SLEDAI), disease damage was quantified by SLICC-Damage Index (SDI). Sera was tested for OxLDL, 8-OHdG, and total antioxidant status (TAS) by double-antibody sandwich ELISA; MDA measured by *Colorimetric assay*. Oxidative stress markers were compared between group1-controls, group 2-mildly active SLE (SLEDAI $\leq$ 5), group 3- moderate to highly active SLE (SLEDAI $\geq$ 6). SLE patients had significantly higher MDA, 8-OHdG and lower TAS when compared to healthy controls, while OxLDL was similar in the three groups. MDA, 8-OHdG were significantly higher, TAS lower in group 3 compared to group 2. MDA had positive correlation with SLEDAI, TAS negatively correlated with SLEDAI. SLE with neuropsychiatric manifestations, vasculitis, anti-sdDNA antibodies had higher MDA, MDA/TAS ratio. SLE patients with thrombocytopenia, and vasculitis had higher OxLDL. Only OxLDL was significantly higher in those patients who have SDI > 1. SLE patients have increased oxidative stress measured by increases in MDA, 8-OHdG, and lower total antioxidant status that was associated with disease activity and some disease manifestations. However only OxLDL was associated with damage.

Keywords Nephritis · Neuropsychiatric lupus · Oxidative stress · Malondialdehyde · Oxidized · LDL

Introduction

Systemic lupus erythematosus (SLE) also known as lupus is the prototype autoimmune disease with multisystem involvement characterised by the production of multiple autoantibodies. An important pathogenetic mechanism of lupus involves delayed clearance of apoptotic bodies potentially exposing intracellular antigens to the immune system and reactive oxygen species (ROS) [1]. In normal conditions mild to moderate levels of reactive oxygen species (ROS) are required for the regulation of cellular processes. But when there is overproduction of ROS or when there is anti-oxidants deficiencies molecules such as lipids, DNA, and proteins get oxidatively modified that can accelerate disease process as has been seen in diabetes and hypertension [2].

In SLE prolonged interaction of the nuclear debris (exposed during apoptosis) and ROS can lead to generation of epitopes with modified antigens, which can cause

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production of autoantibodies leading to further inflammation and organ damage [1]. The mitochondria, blood cells, and vascular endothelium are potential endogenous sources of ROS in lupus. Mitochondrial dysfunction in lupus was shown to cause increased production of ROS, transfer of oxidative stress through bloodstream, with diminished levels of intracellular glutathione (GSH) [3–5]. Experimental studies involving lupus vulnerable mice show that replenishment of glutathione with N-acetylcysteine can reduce lupus disease activity [6]. It is proposed that treatment combination using N-acetylcysteine and hydroxychloroquine can relieve oxidative stress and lupus activity [7–9].

Malondialdehyde (MDA), 4-Hydroxynonenal (4HNE), F2-Isoprostanes are markers of lipid peroxidation that are increased in SLE patients and variably associated with disease activity, nephritis, and atherosclerosis [10–12]. Low density lipoprotein (LDL) can also get oxidised through lipoxygenase or non-enzymatically. Oxidised LDL (OxLDL) formed thereof in the walls of blood vessels is known to accelerate atherosclerosis [13, 14]. 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a product of oxidatively modified DNA base guanine [4]. Though MDA have been studied before in lupus there is limited data on the association of OxLDL and 8-OHdG with lupus disease activity and disease damage. So the objective of this study was to study oxidative stress in SLE, by estimating serum levels of OxLDL, 8-OHdG, MDA, and total anti-oxidant status and to correlate with SLE disease activity and disease damage.

Patients and Methods

This was a cross-sectional study, carried out in the department of Medicine and Clinical Immunology at a tertiary care hospital in southern India from September 2015 to April 2017. Adult SLE patients fulfilling the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) 2012 classification criteria [15] were invited to participate. Those with clinically obvious infections, renal insufficiency (eGFR < 60 ml/min/1.7 m^2), other co-existing connective tissue diseases, drug induced lupus, smoking, and alcohol consumption were excluded. Demographic characteristics and clinical manifestations namely arthralgia/arthritis, mucocutaneous manifestations (malar rash, photosensitivity, oral ulcers, alopecia, and discoid rash), hematological, lupus nephritis, neuropsychiatric manifestations, vasculitis, and gastrointestinal involvement were noted.

Clinical examination included measurement of blood pressure (BP) in mmHg, height (in meters), weight (in kg), and body mass index (BMI) in kg/m^2. Hypertension was defined as BP $> 140/90$ or if patient was on antihypertensives. Diabetes was defined as per the American Diabetes Association Criteria 2012 [16].

SLE disease activity was measured by Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-2K) [17, 18]. SLE disease damage was measured by SLICC/ACR Damage Index (SDI) [19]. Autoantibody profile by semi-quantitative line immunoassay, serum complements, lipid profile was done for most patients. Controls were age and sex matched apparently healthy volunteers who were invited to participate in the study from among blood donors, nursing staff and other hospital staff. The controls were not known to have diabetes, hypertension, autoimmune disease or any chronic illnesses. Physical examination for controls included measurement of height (cm), weight (kg), and BMI. The consensus definition for Asian Indians was used to classify BMI [20].

Five mL venous blood was collected from participants, separated sera was stored at -80 degrees until analysis. Sera was tested for OxLDL, 8-OHdG, MDA, and total antioxidant status. *OxLDL* was measured by *Double antibody sandwich ELISA* using commercial kits (Genxbio, New Delhi, India). Assay range of the kit was 40-100,000 ng/L; *8-hydroxy-2′-deoxyguanosine (8-OHdG)* was measured by *Double antibody sandwich ELISA* using commercial kits (QAYEE-BIO, Shanghai, China). Assay range of the kit 1.25-80 ng/ml; *Malondialdehyde (MDA)* was measured by *Colorimetric assay* using commercial kits Quantichrom™ TBARS assay Kit (BioAssay Systems, Haywart, CA 94545, USA). Assay range of the kit 1-30 μM; *Total antioxidant capacity* was measured by *Double antibody sandwich ELISA* using commercial kits (Bioassay Technology Lab, Shanghai, China). Assay range of the kit 0.3–90 Units/ml.

Oxidative stress biomarkers were compared between three groups: (1) SLE with mild disease activity i.e. SLEDAI score ≤ 5, (2) SLE with moderately active disease and above, i.e. SLEDAI score of ≥ 6, (3) Healthy controls.

Sample size for control group was 80 and SLE 80, estimated based on the minimum expected difference in MDA between study groups as 1.3 μM with SD of 2.6. The sample size was estimated at 5% level of significance and 90% power.

Statistical Analysis

Comparison of oxidative biomarkers between healthy controls, mildly active SLE, and moderate to highly active SLE was done by Kruskal–Wallis Test. Correlation of oxidative biomarkers with SLEDAI score was done by Spearmann's Rank correlation. The comparison of oxidative stress markers with lupus disease variables was done by Mann–Whitney U test. The comparison of oxidative stress markers with presence or absence of hypertension,

Table 1 Baseline clinical characteristics of SLE patients

Characteristic	SLE (n = 80)
Duration of disease, months, median (IQR)	36 (12–72)
BMI kg/m^2 (Mean ± SD)	23.19 ± 5.33
SBP, mean ± SD	119.8 ± 22.94
DBP, mean ± SD	77.05 ± 11.04
Malar rash	52 (65)
Discoid rash	21 (26.25)
Photosensitivity	58 (72.5)
Alopecia	61 (76.25)
Oral ulcers	56 (70)
Serositis	14 (17.5)
Arthralgia or arthritis	69 (86.25)
Lupus Nephritis	36 (45)
Neuropsychiatric manifestations	31 (38.8)
Left ventricular ejection fraction < 50% (data for 78)	5 (6.4)
Vasculitis	8 (10)
Gastrointestinal involvement	3 (3.75)
Leukopenia (Total Leukocyte Count < 4000/mm^3)	23 (28.8)
Thrombocytopenia (platelet count < 1,00,000/mm^3)	18 (22.5)
Autoimmune haemolytic anemia	12 (15)
Antiphospholipid syndrome	4 (5)
SLEDAI mean (SD)	9.66 ± 6.85
SLICC/ACR Damage Index, median (IQR)	0 (0-1)
Hypertension	16 (20)
Diabetes mellitus	11 (13.75)
Hypothyroidism	19 (46.34)

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *SLEDAI* systemic lupus erythematosus disease activity index; Systemic Lupus International Collaborating Clinics/American College of Rheumatology damage index

diabetes, auto-antibody and lipid profile parameters was also done by Mann–Whitney U test. All analysis was done at 5% level of significance and p values < 0.05 considered significant. All patients gave written informed consent, and the study protocol was approved by the Institute Ethics committee (Human Studies) letter no JIP/IEC/2015/17/648, 22-07-2015.

Results

Eighty SLE patients with mean age 30.88 ± 10 years were studied, 90% were women. The mean age of controls was 31.34 ± 10.13 years, and 90% of participants in the control group also were women. The baseline characteristics of SLE patients are given in Table 1. Major organ involvement was lupus nephritis seen in 36 (45%), 17 of 36 had class IV nephritis. Thirty-one (38.8%) had neuropsychiatric SLE (NPSLE). Fourteen of 56 (25%) had antiphospholipid antibody (aPL) positivity either anticardiolipin or anti-beta2 glycoprotein1 or lupus anticoagulant. Only 4 out of 56 (7.14%) fulfilled the classification criteria for antiphospholipid syndrome. At the time of enrolment 77 patients had disease activity (Table 2). Forty-four of 80 (55%) had no disease damage (SDI of 0), 27 (33.8%) had a SDI of 1, 8 (10%) had a SDI of 2, only one patient had SDI of 3.

Eight (10%) were underweight (BMI < 18 kg/m^2), BMI was normal (18-22.9) in 32 (40%); 13 (16.25%) were overweight (BMI of 23–24.9), 27 (33.75%) were obese (BMI > 25). Fifteen of 80 patients (18.75%) had impaired fasting glucose, while 11 (13.75%) had diabetes. Almost 35% i.e. 23/66 individuals had hypercholesterolemia (total cholesterol > 200 mg/dL), 45% i.e. 34/66 had hypertriglyceridemia (triglycerides > 150 mg/dL), and 62% i.e. 40 out of 64 had HDL cholesterol < 50 mg/dL.

Autoantibody profile were available for 66 patients; 47 of 66 (71.2%) were positive for anti-dsDNA, 43.4% were positive for anti-SSA, 13% positive for anti-SSB, 43.4% positive for anti-nucleosome, and 28.3% were positive for anti-Sm antibodies. Forty-two of 57 (73%) had low C3, and 31/55 (56.3%) had low C4. All patients were on hydroxychloroquine and prednisolone. Forty-four patients have received intravenous cyclophosphamide, thirteen patients were on azathioprine, and eight were on mycophenolate mofetil.

Oxidative Stress

SLE patients had significantly higher levels of MDA, 8-OHdG and lower TAS when compared to healthy controls, while OxLDL was similar in the three groups (Table 3). Both MDA, 8-OHdG were significantly higher and TAS lower in those with moderate to high disease activity compared to those with low disease activity. The ratio of MDA/TAS was highest in SLE patients with moderate to very highly active disease (Table 3).

Oxidative Stress and SLE Disease Activity

MDA and MDA/TAS ratio had good positive correlation with SLEDAI, while TAS negatively correlated with SLEDAI (Table 4). There was no correlation of OxLDL or 8-OHdG with SLEDAI.

Oxidative Stress and SLE Disease Variables

The levels of OxLDL, MDA, 8-OHdG, TAS, MDA/TAS ratio was compared with presence or absence of lupus disease variables namely nephritis, NPSLE, musculoskeletal, mucocutaneous, vasculitis, hematological, leukopenia, and thrombocytopenia. Positive findings are summarised as follows:

Table 2 SLEDAI at the time of enrolment into study

SLE disease activity	SLEDAI score	No of patients (%)
No disease activity	0	3 (3.75)
Mild	1–5	24 (30)
Moderate	6–10	22 (27.5)
High	11–19	29 (36.25)
Very high	≥ 20	2 (2.5)

SLEDAI systemic lupus erythematosus disease activity index

OxLDL was significantly higher in *SLE with vasculitis* compared to those without vasculitis; OxLDL (median) was 8324.23 ng/L (range 1798–28650) versus 2267.50 ng/L (range 503–28752) in vasculitis and without vasculitis, respectively, $p = 0.035$. OxLDL was also significantly higher in *SLE with thrombocytopenia* compared to those without thrombocytopenia; OxLDL (median) was 5079.15 ng/L (range 503–28650) versus 2237.81 ng/L (range 671–28752) in SLE with and without thrombocytopenia respectively, $p = 0.027$.

MDA was significantly higher in *NPSLE* than in those without NPSLE. MDA (median) was 2.49 μM (range 1–8) versus 2.20 μM (range 1–8) respectively, $p = 0.03$.

TAS was significantly lower in those with *NPSLE* compared to non-NPSLE; TAS (median) was 12.08 units/L (range 0–18) and 14.35 units/L (range 0–20) in NPSLE and non-NPSLE respectively, $p = 0.003$. Those patients with *vasculitis* had significantly lower TAS compared to those without vasculitis. TAS (median) was 10.72 U/L (range 0–13) versus 14.35 U/L (IQR 0–20) in those with and without vasculitis respectively, $p = 0.001$. So also, the ratio of MDA/TAS was significantly higher in those with *NPSLE* ($p = 0.005$), and vasculitis (0.025).

8-OHdG- No association of 8-OHdG was found with SLE variables.

Oxidative Stress and SLE Disease Damage

Table 5 shows that OxLDL emerged significantly higher in those patients who have disease damage. However MDA, 8-OHdG, TAS, MDA/TAS were comparable in those with and without disease damage.

Oxidative Stress and Autoantibodies

The levels of OxLDL, MDA, 8-OHdG, TAS, MDA/TAS with respect to antibodies to SSA, SSB, Sm, nucleosome, and dsDNA are given in Table 6. Positive anti-dsDNA antibodies status is associated with significantly higher MDA levels, lower TAS, and higher MDA/TAS ratio. The ratio of MDA/TAS is also significantly higher in those with anti-SSB positivity.

Oxidative Stress and Metabolic Variables

The levels of OxLDL, MDA, 8-OHdG, TAS, MDA/TAS were not significantly different in patients with and without diabetes, hypertension, hypercholesterolemia, hypertriglyceridemia, and low HDL-Cholesterol. There was also no correlation of systolic BP, diastolic BP, and BMI with any of the oxidative stress parameters.

Table 3 Comparison of oxidative stress biomarkers between healthy controls, mildly active SLE, and moderate to highly active SLE

Oxidative stress biomarker	Group 1 Healthy controls N = 80	Group 2 Low disease activity (SLEDAI ≤ 5) N = 27	Group 3 Moderate–High disease activity (SLEDAI ≥ 6) N = 53	Statistical significance *p* value
OxLDL (ng/L)	2605.68 (135–32,345)	2494.76 (671–28,752)	2586.55 (503–28,650)	0.726
MDA (μM)	0.50 (0–4)	1.73 (1–2)	2.91 (1–8)	< 0.001
8-OHdG (ng/mL)	17.28 (0–151)	18.53 (0–304)	20.12 (0–725)	< 0.05
Total antioxidant status (TAS) (units/mL)	23.77 (0–234)	17.44 (0–20)	12.02 (0–16)	< 0.001
MDA/TAS ratio	0.015 (0.0–1.13)	0.10 (0.05–1.90)	0.30 (0.08–7.89)	< 0.001

Statistical test used was Kruskal–Wallis Test, Values are median (IQR)

SLEDAI systemic lupus erythematosus disease activity index, *OxLDL* Oxidised LDL, *MDA* Malondialdehyde, *8-OHdG* 8-hydroxy-2′-deoxyguanosine, *TAS* total antioxidant status

Table 4 Correlation of oxidative stress parameters with disease activity (SLEDAI)

SLEDAI	Correlation co-efficient (Spearman's rho)	*p*
SLEDAI versus OxLDL	− 0.039	0.731
SLEDAI versus MDA	0.738	< 0.001
SLEDAI versus 8-OHdG	− 0.082	0.45
SLEDAI versus TAS	− 0.588	< 0.001
SLEDAI versus MDA/TAS	0.647	< 0.001

Statistical test used Spearman's Rank correlation

SLEDAI systemic lupus erythematosus disease activity index, *OxLDL* Oxidised LDL, *MDA* Malondialdehyde, *8-OHdG* 8-hydroxy-2′-deoxyguanosine, *TAS* total antioxidant status

Discussion

The marker for oxidative stress used in majority studies of oxidative stress in SLE is serum or plasma MDA [21–26]. Though these studies showed that MDA is present in excess in SLE, not all confirmed association with disease activity (SLEDAI). We also confirmed that SLE patients have reduced total antioxidant status (TAS), elevated MDA and 8-OHdG (but not OxLDL). This was more so in those having a higher SLEDAI. On correlation analysis there was good positive correlation of MDA and negative correlation of TAS with SLEDAI.

In line with our findings Hassan et al. found positive correlation of MDA with SLEDAI, and negative correlation of GSH and GSH peroxidase with SLEDAI [21]. They also found no association of oxidative markers with SLE damage index. Similarly, Turgay et al. in a study of 28 SLE (active n = 16, inactive n = 12) observed that plasma MDA was higher in active lupus compared to inactive lupus [22]. Shah et al. in studies of oxidant and anti-oxidant enzymes on erythrocyte hemolysate documented increases in plasma MDA in SLE patients, decreased levels of anti-oxidant enzymes, and increased levels of IFN-gamma, IL-12 in peripheral blood mononuclear cells (PBMC) of SLE patients [23]. Further there was positive correlation of plasma MDA with SLE disease activity, IFN-gamma, and IL-12. This imbalance of oxidative stress and pro-inflammatory Th1 cytokine probably contribute to heightened SLE disease activity.

There are studies that show association of disease activity with reduced levels of anti-oxidant enzymes but not with MDA. The study by Tewthanom et al. reported that plasma MDA levels did not differ among mild or moderate or severe SLE from the control group, but there was a significant negative correlation between plasma GSH and SLE severity [24]. Su et al. studied the association of RBC antioxidant enzymes, autoantibodies, and disease severity in SLE (n = 32) and controls (n = 16) found that superoxide dismutase was lower in SLE but no association with SLEDAI [25]. There are negative studies as well. Pérez et al. have not identified in their study the association of MDA or sulfhydryl (SH) levels with SLEDAI scores [26]. Overall there is evidence that oxidative stress is increased in lupus patients and this is possibly contributing to drive excess disease activity.

Oxidative stress in lupus is associated with individual manifestations such as nephritis, hypertension, etc. [10, 11, 27]. Avalos et al. in their study of 58 SLE and 105 controls found that urinary F2-isoprostane excretion was associated with fatigue and pain but neither with SLEDAI nor with damage [10]. Dwiwedi et al. found a significantly decreased level of the serum TAS, plasma vitamin C level, and increased serum MDA in lupus nephritis [27]. In our study we found that MDA was higher and TAS lower among those SLE patients who had neuropsychiatric

Table 5 Comparison of oxidative stress biomarkers of SLE patients with and without disease damage

Oxidative stress biomarker	SLE disease damage absent (SDI 0) N = 44	SLE disease damage present (SDI ≥ 1) N = 36	Statistical significance *p* value
OxLDL (ng/L)	2202.86 (502.7–28751.8)	3687.17 (670.9–28649.7)	0.03
MDA (μM)	2.36 (0.83–7.90)	2.28 (1.03–7.61)	0.85
8-OHdG (ng/mL)	19.06 (0.00–538.77)	20.39 (0.00–724.52)	0.10
TAS (units/mL)	13.15 (4.70)	11.44 (6.54)	0.2
MDA/TAS	0.18 (0.05–7.89)	0.23 (0.06–7.60)	0.41

All values are median (IQR)

OxLDL Oxidised LDL, *MDA* Malondialdehyde, *8-OHdG* 8-hydroxy-2′-deoxyguanosine, *TAS* total antioxidant status, *SDI* SLE damage index

Table 6 Comparison of OxLDL, 8-OHdG, MDA, total antioxidant status, MDA/TAS in relation to autoantibody status

	Autoantibody positive	Autoantibody negative	*p*
OxLDL*			
SSA (N = 53)	23	30	0.733
OxLDL	2207.46 (503–17665)	2167.00 (905–28752)	
SSB (N = 53)	7	46	0.813
OxLDL	2586.55 (1192–17665)	2166.68 (503–28752)	
Sm (N = 53)	15	38	0.236
OxLDL	1797.63 (503–17703)	2253.28 (906–28752)	
Nucleosome (N = 53)	23	30	0.720
OxLDL	2119.42 (503–17703)	2253.28 (905–28752)	
ds-DNA (N = 66)	47	19	0.388
OxLDL	2785.06 (503–28650)	2119.42 (671–27103)	
8-OHdG*			
SSA (N = 53)	23	30	0.518
8-OHdG	17.57 (0–241)	18.73 (13–344)	
SSB (N = 53)	7	46	0.563
8-OHdG	19.6 (13–241)	18.56 (0–344)	
Sm (N = 53)	15	38	0.441
8-OHdG	17.51 (0–344)	18.64 (0–304)	
Nucleosome (N = 53)	23	30	0.647
8-OHdG	19.6 (0–344)	18.43 (0–304)	
ds—DNA (N = 66)	47	19	0.804
8-OHdG	19.60 (0–725)	18.79 (16–142)	
MDA*			
SSA (N = 53)	23	30	0.49
MDA	2.49 (1–8)	2.21 (1–8)	
SSB (N = 53)	7	46	0.134
MDA	3.08 (2–8)	2.23 (1–8)	
Sm (N = 53)	15	38	0.790
MDA	2.23 (1–8)	2.41 (1–8)	
Nucleosome (N = 53)	23	30	0.936
MDA	2.23 (1–8)	2.41 (1–8)	
ds-DNA (N = 66)	47	19	0.003
MDA	2.53 (1–8)	1.93 (1–5)	
TAS*			
SSA (N = 53)	23	30	0.666
TAS	14.48 (0–18)	13.30 (0–18)	
SSB (N = 53)	7	46	0.012
TAS	7.10 (0–15)	14.36 (0–18)	
Sm (N = 53)	15	38	0.244
TAS	13.23 (0–18)	14.42 (0–18)	
Nucleosome (N = 53)	23	30	0.986
TAS	14.35 (0–18)	13.30 (0–18)	
ds-DNA (N = 66)	47	19	0.004
TAS	12.9 (0–18)	17.22 (0–18)	
MDA/TAS*			
SSA (N = 53)	23	30	0.97
MDA/TAS	0.20 (0.05–7.89)	0.22 (0.05–4.75)	
SSB (N = 53)	7	46	0.026
MDA/TAS	0.43 (0.14–7.60)	0.17 (0.05–7.8)	

Table 6 continued

	Autoantibody positive	Autoantibody negative	*p*
Sm (N = 53)	15	38	0.95
MDA/TAS	0.17 (0.05–7.89)	0.21 (0.05–7.60)	
Nucleosome (N = 53)	23	30	0.97
MDA/TAS	0.17 (0.06–4.75)	0.24 (0.05–7.89)	
ds-DNA (N = 66)	47	19	0.06
MDA/TAS	0.27 (0.05–7.89)	0.12 (0.05–2.24)	

OxLDL oxidised LDL, *8-OHdG* 8-hydroxy-2′-deoxyguanosine, *MDA* malonaldehyde, *TAS* total antioxidant status

Units of measurements—OxLDL is in ng/L, 8-OHdG is ng/ml, MDA is μM, TAS is U/mL

*All values are median (IQR)

manifestations (NPSLE). The relationship of OxLDL, MDA, 8-OHdG and TAS with other lupus manifestations was not significant because of small sample size. It is also possible that the lack of association of oxidative stress biomarkers with a major organ involvement such nephritis, was because patients were on treatment, moreover SLE is a multisystem disease and different patients have variable degree of organ involvement. Immunosuppressive therapy can influence the levels of oxidative biomarkers.

The second observation from our study was decreased 8OHdG in highly active SLE compared to those with mildly active SLE. Previous workers (Lee et al.) had shown that plasma 8OHdG was significantly low in SLE patients along with decreases of leukocyte anti-oxidant enzymes [28]. 8OHdG is a marker of oxidatively modified DNA. A damage in DNA would cause increased binding of anti-DNA antibodies to the denatured DNA and predispose aberrant autoantibodies formation to oxidized protein or lipid or DNA [29–31]. It has been shown by Wang et al. that SLE patients have high levels of autoantibodies to lipid peroxidation products namely MDA, 4-hydroxynonenal (HNE), and MDA/HNE protein adducts [32]. The level of these antibodies are higher in those with SLEDAI > 6, suggesting a ROS driven production of antibodies resulting in further inflammation and disease activity.

The third observation of our study was significantly higher MDA levels and lower TAS in anti-dsDNA antibody positive and anti-SSB antibody positive patients. Anti-dsDNA antibodies are known to be associated with active SLE. Similarly anti-SSB antibodies are also specific diagnostic marker for SLE, and their presence is associated with mucocutaneous manifestations, serositis, secondary sjogrens, and leukopenia [33]. Therefore this association of anti-dsDNA and anti-SSB antibodies with high MDA and lower TAS might be a reflection of higher oxidative stress in active lupus. Su et al. in their study of 32 patients found positive correlation of anti-Sm antibodies (which is a specific immunological marker of SLE) with oxidative stress [25].

One large study (n = 147) found higher OxLDL in SLE with nephritis, leukopenia, and arterial disease, but OxLDL levels were not associated with activity nor damage [34]. In our case OxLDL was associated with vasculitis and thrombocytopenia, and OxLDL was significantly higher in those having disease damage. The SLE damage index is a measure of irreversible complication during the course of lupus examples being a cataract or a stroke or a renal failure [19]. Though OxLDL is a surrogate marker of atherosclerosis (and cardiovascular disease) in general population and SLE as well [12, 13, 35], we found no association with diabetes, BMI etc. because of limited sample size. Moreover harder end points of premature atherosclerosis namely the thickness of intima media thickness was not assessed.

The implications of oxidative stress is that anti-oxidants have potential role in the supportive treatment in of SLE. Depletion of Intracellular glutathione activates the mechanistic target of rapamycin (mTOR). In turn mTOR is known to drive an imbalance in T cell distribution in SLE, secretion of inflammatory cytokines and abnormalities of autophagy [36, 37]. Thus N-acetylcysteine a precursor of glutathione was tested in two randomised trials and was found to ameliorate lupus disease activity [9, 38], improvement in endothelial function [39], and nephritis [7]. Rapamycin the inhibitor of mTOR is also suggested as a treatment option [36]. Vitamin E treatment in SLE patients cause significant reduction in anti-dsDNA titers, but no change in urinary 8OHdG [40]. It is suggested that Vitamin E as an antioxidant reduces autoantibody production in SLE. Other promising therapeutic strategies include activation of endogenous anti-oxidant enzymes and development of more potent free radical scavengers and elimination of source of ROS [41]. Lupus patients benefit by avoiding exposure to direct sunlight as UV light is a known trigger of generation of ROS.

The limitations of our study was that intracellular glutathione levels which represents the major anti-oxidant defence system was not studied. Secondly the study was

not powered to detect differences of these biomarkers in major disease manifestations subgroups namely lupus nephritis and neuropsychiatric SLE. Further the robustness of our findings would have been better if we had included a disease control group.

To conclude lupus patients have increased oxidative stress measured by increases in MDA, 8-OHdG, and a lower total antioxidant status that was associated with overall disease activity and some disease manifestations such as vasculitis, thrombocytopenia, and neuropsychiatric manifestations. However only OxLDL was associated with disease damage.

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Compliance with Ethical Standards

Conflict of interest All the authors declare that they have no conflict of interest.

Ethical Approval All procedures in the study were carried out according to the World Medical Association principles as laid out in the Declaration of Helsinki. The project was approved by the institute ethics committee before the 1st participant was recruited.

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