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RESEARCH ARTICLE

Association between T lymphocyte sub-sets apoptosis and peripheral blood mononuclear cells oxidative stress in systemic lupus erythematosus

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Abstract
Increased oxidative stress and lymphocyte apoptosis are a hallmark of the autoimmune disease systemic lupus erythematosus (SLE). However, the association between oxidative stress and T lymphocytes apoptosis has still to be elucidated in SLE. In order to appraise the interaction between oxidative stress and T lymphocyte apoptosis with the severity of disease, oxidative stress profile and T lymphocytes apoptosis were studied. Increased levels of ROS, MDA and $CD4^+$ lymphocyte apoptosis were positively associated with disease activity while decreased levels of GSH and percentage expression of $CD4^+$ lymphocyte were negatively associated with disease activity. The decrease in intracellular levels of GSH was negatively associated with T lymphocyte, $CD4^+$ lymphocyte, $CD8^+$ lymphocyte apoptosis and intracellular caspase-3 expression. The present study suggests that increased T lymphocyte sub-sets apoptosis may be mediated by decreased intracellular glutathione concentration and severity of disease might be enhanced together by over-production of ROS in SLE.

Keywords: *Reactive oxygen species, redox system, T lymphocyte sub-sets, apoptosis, systemic lupus erythematosus*

Abbreviations: *SLE, systemic lupus erythematosus; ROS, reactive oxygen species; SLEDAI score, systemic lupus erythematosus Disease Activity Index score; GSH, reduced glutathione; LPO, lipid peroxidation; MDA, malondialdehyde; DCFH-DA, 2', 7'-dichlorofluorescein diacetate; PBMC, peripheral blood mononuclear cells; FITC, fluorescein isothiocyanate; PI, propidium iodide; MFI, mean fluorescence intensity; ESR, erythrocyte sedimentation rate; C3, complement component 3; C4, complement component 4*

Introduction

Systemic lupus erythematosus (SLE) is a multifactorial autoimmune disease characterized by the presence of autoantibodies, especially against nuclear components. The assortment of autoantibodies produced is broad and as a consequence the manifestations of the disease are diverse. Certain long-term complications are quite common; these include renal, cardiovascular, neuropsychiatric and musculoskeletal damage [1]. A key issue in the pathogenesis of lupus is how intracellular antigens become exposed and targeted by the immune system [2]. In this regard, excessive ROS production, redox imbalance [3] and a defect in apoptosis [4] are considered as imperative factors involved in the development of antibody flares and various clinical features in SLE. Reactive oxygen species generated by phagocytic cells may cause damage to DNA and other macromolecules and autoantibodies to a self-antigen are produced. Recently, the role of ROS and redox balance in the perturbation of cell death by apoptosis and necrosis has gained deep attention in autoimmune diseases such as SLE [5,6]. A defect in control of apoptosis or programmed cell death and delayed clearance of apoptotic cells may provide sustained interaction between reactive oxygen species and apoptotic cell macromolecules generating neoepitopes which subsequently result in autoantibody formation [7].

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Excessive ROS production disturbs redox status, damages macromolecules, including DNA and can modulate expression of a variety of immune and inflammatory molecules leading to inflammatory processes, exacerbating inflammation and affecting tissue damage [8]. The primary targets of ROS are double bonds in polyunsaturated fatty acids in the cell membrane, which increase lipid peroxidation (LPO) and result in more oxidative damage [9,10]. Additionally, oxidative damage mediated by ROS resulting in generation of deleterious by-products, such as aldehydic products, lead to the formation of adducts with proteins that in turn make them highly immunogenic, thus inducing pathogenic antibodies leading to tissue damage in SLE [11]. An increase in MDA-modified proteins, an anti-SOD and anti-catalase antibody in the sera of SLE patients reinforces the evidence of oxidative stress in the disease [12,13]. There has been well documented the role of low glutathione in the perturbation of apoptosis including the complement protein in autoimmune disease [14]; however, the inter-play of ROS, redox system and apoptosis together in the pathogenesis of SLE has still to be illuminated.

Thus, the aim of this study was to explore relationships between ROS, redox system and T lymphocyte sub-sets apoptosis with respect to the disease severity of SLE, which may have further implications in understanding SLE pathology and therapeutic management of the disease.

Materials and methods

Patients and controls

Patients for the study were selected from individuals attending out-patient Department of Internal Medicine at Postgraduate Institute of Medical Education and Research, Chandigarh, India. The study included 35 patients with SLE (30 females, five males) with mean age of 28.5 ± 7.80 years and the control group consisted of 35 healthy volunteers (30 females, five males) with mean age of 28.25 ± 6.80 years. The disease was diagnosed using the American College of Rheumatology (ACR) 1997 revised criteria [15]. Disease activity was determined using SLE Disease Activity Index (SLEDAI) score [16]. The study protocol was approved by the Institute Ethics Committee, Postgraduate Institute of Medical Education and Research, Chandigarh, India and informed consent was obtained from all the patients and healthy subjects. All the patients enrolled in the present study were non-smokers and non-alcoholics, not associated with any other autoimmune disease and were not undergoing any anti-inflammatory therapy.

Blood samples

Venous blood samples obtained from patients and controls were collected into heparinized vacutainers (Becton Dickinson, San Jose, CA, USA). Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by density gradient centrifugation using Ficoll-Histopaque-1077 (Sigma-Aldrich, St. Louis, MO, USA) [17] for measuring oxidative stress profile, percentage T lymphocyte sub-sets and apoptosis of T lymphocyte sub-sets.

Determination of ROS

The PBMCs isolated from the blood samples were washed twice in phosphate-buffered saline (PBS) and requisite cell numbers were enumerated using a haemocytometer and cell viability was assessed by trypan blue exclusion method. ROS production was detected using the dye 2',7'-dichlorofluorescein diacetate (DCFH-DA, Sigma) according to the method of Sarkar et al. [18]. In brief, PBMC (1×10^6 cells/ml) were incubated at 37°C for 30 min with 2',7'-dichlorofluorescein diacetate (10 μM) in dark. Cells were then washed, resuspended in PBS and kept on ice for an immediate detection of ROS by flow cytometry (FACS Calibur, Becton Dickinson, San Jose, CA) with excitation and emission setting of 488 and 530 nm, respectively. The median of the FL-1 channel of fluorescence was used as the parameter to evaluate the intracellular content of ROS because it matches the maximal number of cells with the highest fluorescence.

Lipid peroxidation

The quantitative measurement of lipid peroxidation (LPO) in the term of MDA equivalent was performed in the PBMCs according to the method of Ohkawa et al. [19]. The amount of MDA formed was measured by the reaction with thiobarbituric acid at 532 nm. The results are expressed as nmol MDA/mg protein using molar extinction coefficient of MDA-thiobarbituric chromophore (1.56×10^5 $M^{-1}cm^{-1}$).

Reduced glutathione

The reduced glutathione (GSH) content was determined by the method described by Tietze [20]. In brief, PBMCs (2×10^6 cells) were resuspended in 100 μl of 4.5% 5-sulphosalicylic acid. The acid-precipitated protein was pelleted by centrifugation at 4°C for 10 min at 2000 × g. The total protein content of each sample was determined using the Lowry et al. [21] assay. GSH content of the aliquot assayed was determined in comparison to reference curves generated with known amounts of GSH.

Determination of $CD4^+$ and $CD8^+$ T lymphocytes

The percentage expression of $CD4^+$ and $CD8^+$ T lymphocytes in PBMCs were carried out by the cell

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surface labelling with mouse anti-human anti-CD4-FITC (Becton Dickinson, San Jose, CA, USA) and mouse anti-human anti-CD8-APC (Immunostep, Salamanca, Spain). The labelled cells were analysed by flow cytometry. In brief, PBMCs (1×10^6 cells/ml) were stained using 20 μl of mouse anti-human anti-CD4-FITC and 20 μl of mouse anti-human anti-CD8-APC and incubated in the dark at 4°C for 1 h. After washing twice with PBS, cells were fixed with 1% paraformaldehyde and incubated at 4°C for half an hour. After washing twice with PBS, cells were resuspended in 300 μl of PBS. The negative controls were performed simultaneously using FITC-labelled mouse anti-human IgG1 mAb (BD Bioscience) and APC-labelled mouse anti-human IgG1 mAb (BD Bioscience). Cells were gated for lymphocyte characteristics using both forward and side scatter and the expression of $CD4^+$ and $CD8^+$ T cells were quantified by acquiring 10 000 cells on a flow cytometer (FACS-Calibur, Becton Dickinson) using Cell Quest software (Becton Dickinson).

Determination of T lymphocyte sub-sets apoptotic cell population

In lymphocytes, the phosphatidylserine (PS) is located on the inner leaflet of the plasma only. During apoptosis, translocation of PS from the inner to the outer leaflet is one of the earliest detectable features of cells undergoing apoptosis. Fluorescein isothiocynate (FITC)-Annexin V and propidium iodide (PI) double staining of the cells can differentiate early apoptotic cells (FITC-Annexin positive, PI negative) and late apoptosis or necrotic cells (FITC-Annexin positive, PI positive). The percentages of apoptotic cells and necrotic cells were determined by flow cytometry using a commercially available apoptosis detection kit (Immunostep). In brief, 5 μL each of Annexin V-FITC and propidium iodide (PI) was added to the PBMC incubated with mouse anti-human anti-CD3-PE-Cy5 (BD Bioscience), mouse anti-human anti-CD4-PE-Cy5 (BD Bioscience) and mouse anti-human anti-CD8-APC (BD Bioscience) and then resuspended in 500 μL of binding buffer and incubated at room temperature for 15 min. The cells were analysed by FACSCalibur (Becton Dickinson) where the cells were gated for lymphocyte characteristics using both forward and sideward scatter. The cells populations were quantified by acquiring 10 000 cells for each sample and data was analysed using the CELL Quest program (Becton Dickinson).

Intracellular caspase-3 expression

Intracellular expression of caspase-3 activity was determined by using fluorochrome tagged monoclonal antibody against caspase-3. Apoptosis was induced by H_2O_2 (200 μM) in PBMCs (4×10^6/ml) pre-incubated with mouse anti-human anti-CD3-PE-Cy5 (BD Bioscience) at 37°C in 5% CO_2 atmosphere for 4 h. The cells were harvested after the induction of apoptosis, washed twice with PBS, fixed and permeabilized (250 μl) using the Cytofix/Cytoperm™ Kit (Becton Dickinson) for 20 min at room temperature. After centrifugation (1200 rpm for 5 min) the cells were washed with Perm/Wash™ buffer (Becton Dickinson) and subsequently stained with FITC tagged rabbit anti-human-active caspase-3 antibody for 30 min at room temperature in dark. The cells were washed and active caspase-3 activity was analysed in T lymphocyte by the flow cytometer (FACS-Calibur, Becton Dickinson). Isotype controls and unstained permeabilized cells were used along with the experiments as controls.

Statistical analysis

Statistically significant differences were determined using the Mann-Whitney U-test. Correlation analyses were performed using two-tailed Spearman's rank correlation. Analyses were performed using GraphPad Prizm v.5.00.288 for Windows (GraphPad Software, San Diego, CA). p-values less than 0.05 were considered significant.

Results

The study included 35 patients with SLE (30 females, five males) with mean age of 28.5 ± 7.80 years and the control group consisted of 35 healthy volunteers (30 females, five males) with mean age of 28.25 ± 6.80 years. The demographic and clinical characteristics of SLE patients and healthy controls are summarized in Table I. Disease activity was determined by using SLE Disease Activity Index (SLEDAI) score (maximum score of 105): Mild score <10; Moderate score 10–20; Severe score >20. Six patients had a moderate SLEDAI score while the remaining 34 had a severe SLEDAI score in the present study.

Levels of reactive oxygen species (ROS) in SLE patients and healthy controls

To apprehend the role of mononuclear intracellular ROS in the pathogenesis of SLE, the levels of ROS

Table I. Demographic characteristics of patients with systemic lupus erythematosus (SLE) and controls.

	SLE patients	Controls
Number (*n*)	35	35
Female/male	30/5	30/5
Age (years)	28.5 ± 7.80	28.25 ± 6.80
Duration of disease	2.25 ± 1.50	NA
ESR (mm/h)	47.53 ± 22.50	NA
C3 (mg/dl)	54.20 ± 17.61	NA
C4 (mg/dl)	24.40 ± 12.42	NA
SLEDAI score	34.22 ± 12.26	NA

Values are expressed as Mean ± SD, NA: not applicable.

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were measured in PBMCs of SLE patients and healthy controls. Flow cytometric analysis showed significantly ($p < 0.001$) higher levels of ROS (measured in the term of MFI) production in PBMCs from SLE patients (59.65 ± 16.43) as compared to healthy controls (40.62 ± 9.71) (Table II).

MDA and GSH levels in SLE patients and healthy controls

In order to further explore the role of oxidative stress and redox imbalance in the pathogenesis of SLE, MDA and GSH levels were measured in PBMCs from patients and healthy controls. The levels of MDA and GSH are depicted in Table II. A significant ($p < 0.01$) increase in the level of MDA was observed in SLE patients (0.55 ± 0.15 nmol/mg protein) in contrast to healthy controls (0.40 ± 0.10 nmol/mg protein). Furthermore, the level of antioxidant molecule GSH was remarkably ($p < 0.001$) reduced in SLE patients (247.08 ± 47.97 nmol GSH/mg of protein) when matched with healthy controls (329.27 ± 97.96 nmol GSH/mg of protein).

Percentage expression of $CD4^+$ /$CD8^+$ T lymphocytes in SLE patients and healthy controls

The alteration of $CD4^+$ /$CD8^+$ T cell ratio in patients with SLE has been associated with various clinical features of SLE and may act as a therapeutic marker for the improvement of disease activity in SLE. The percentages of CD4 and CD8 T lymphocytes are depicted in Table II. The percentages of $CD4^+$ T lymphocytes in control and patient samples were 40.29 ± 5.81 and 27.08 ± 6.00, respectively. The $CD8^+$ T lymphocytes were found to be 25.03 ± 3.42% in healthy controls and 36.17 ± 6.49% in SLE patients. Furthermore, $CD4^+$ /$CD8^+$ T cell ratios were 1.60 ± 0.18% in healthy controls and 0.72 ± 0.12% in SLE patients. A significant ($p < 0.001$) increase in the level of $CD8^+$ T cells, a decline in the level of $CD4^+$ T cells, with a consequent reduction in the level of $CD4^+$ /$CD8^+$ T cell ratio, were found in SLE patients as compared to healthy controls.

Table II. Erythrocytes oxidant and antioxidant parameters and lymphocyte apoptosis in patients with systemic lupus erythematosus (SLE) and healthy controls.

Parameters	Controls (n = 35)	SLE patients (n = 35)
ROS (MFI)	40.62 ± 9.71	59.65 ± 16.43***
MDA (nmol/mg protein)	0.40 ± 0.10	0.55 ± 0.15**
GSH (nmol GSH/mg of protein)	329.27 ± 97.96	247.08 ± 47.97***
%CD4 lymphocytes	40 ± 5.88	26.51 ± 7.18***
%CD8 lymphocytes	25.06 ± 3.38	36.19 ± 6.51***
%CD4/CD8 lymphocytes	1.60 ± 0.18	0.72 ± 0.12***
%Caspase-3 activity	20.85 ± 8.34	34.26 ± 13.07***
%T lymphocytes apoptosis	13.49 ± 2.14	22.23 ± 4.75***
%T lymphocytes necrosis	0.95 ± 0.59	2.55 ± 1.11***
%CD4 lymphocytes apoptosis	12.00 ± 4.42	21.16 ± 6.53***
%CD8 lymphocytes apoptosis	15.13 ± 6.46	19.68 ± 7.40**

Values are expressed as Mean ± SD. **$p < 0.010$, ***$p < 0.001$.

Apoptotic cell population in SLE patients and healthy controls

To investigate and differentiate the susceptibility of total T lymphocytes and T lymphocytes sub-sets towards the apoptosis and necrosis, a double staining reagent kit (Annexin V/Propidium Iodide) was used and cells were analysed flow cytometrically. The values of apoptosis and necrosis in the total T lymphocytes and T lymphocyte sub-sets are depicted in Table II. The percentage of early apoptosis in T lymphocyte was significantly ($p < 0.001$) higher in SLE patients (22.23 ± 4.75) than healthy controls (13.49 ± 2.14). Moreover, the percentage of necrotic cells was notably ($p < 0.001$) higher in patient samples (2.55 ± 1.11) as compared to healthy controls (0.95 ± 0.59). The percentage of CD4 T lymphocyte apoptosis (21.16 ± 6.53) was remarkably higher ($p < 0.001$) than healthy controls (12.00 ± 4.42). Further, the percentage of CD8 T lymphocyte apoptosis was significantly ($p < 0.01$) higher in SLE patients (19.68 ± 7.40) as compared to healthy controls (15.13 ± 6.46).

Enhanced expression of intracellular caspase-3 in SLE patients

To test the consequence of amplified apoptosis in SLE patients, we studied the expression of activated caspase-3 in T lymphocyte and assessed them with those of healthy controls. As shown in Table II, the expression rates of intracellular activated caspase-3 in T lymphocyte of SLE patients were significantly ($p < 0.001$) higher (34.26 ± 13.07) when put side-by-side with the healthy controls (20.85 ± 8.34).

Correlation studies

To evaluate the relationship between mononuclear cells oxidative stress, redox system and lymphocytes apoptosis in SLE patients, correlations among the oxidative stress markers and apoptosis were carried out. Further, to appraise together the role of mononuclear cells oxidative stress, redox system and apoptosis with severity of SLE, correlations among oxidative stress markers, apoptosis and disease activity were studied. The disease activity measured as SLEDAI score was positively associated with the levels of ROS ($r = 0.436$, $p < 0.01$, Figure 1A) and MDA ($r = 0.410$, $p < 0.05$, Figure 1B) while negatively associated with the levels of GSH ($r = -0.451$, $p < 0.01$, Figure 1C). A significant

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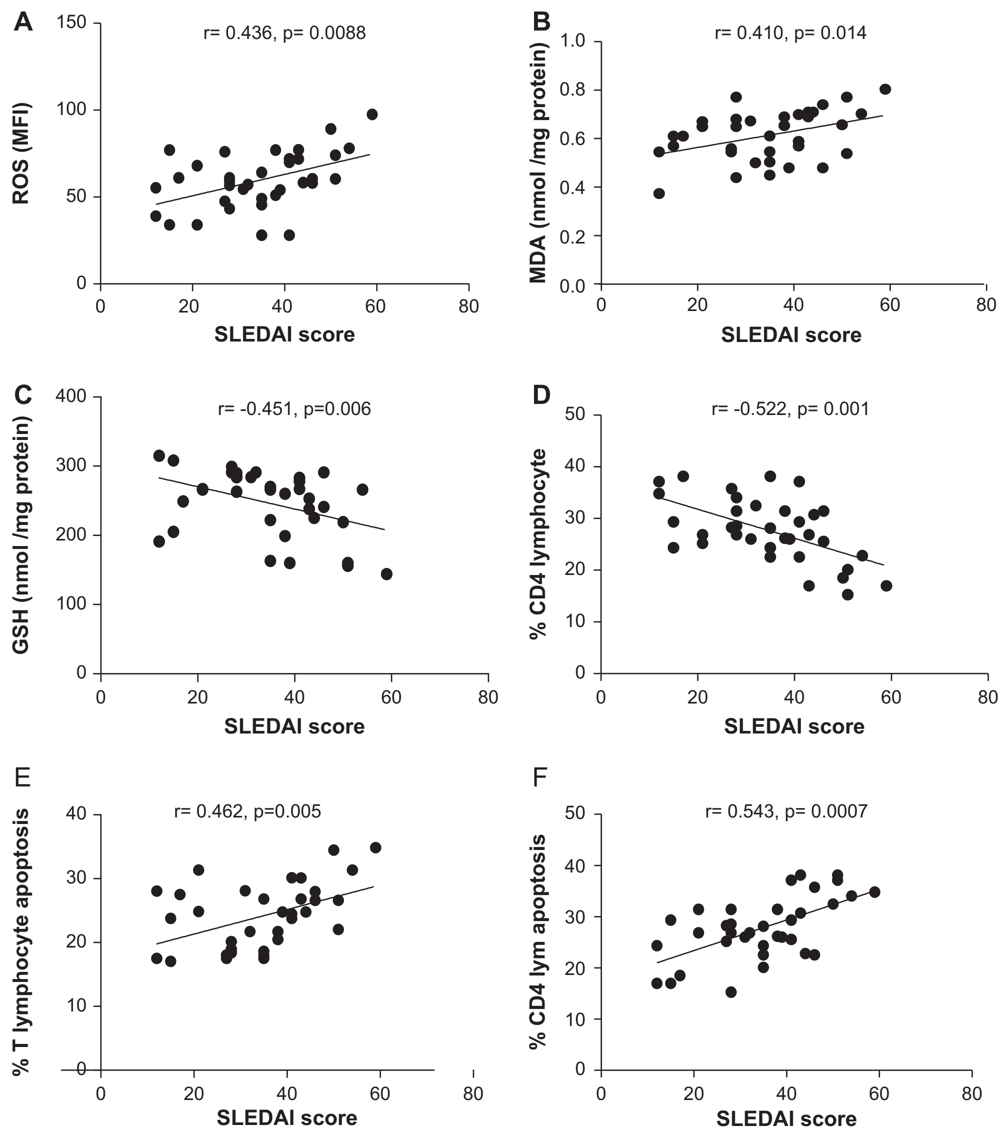

Figure 1. Correlation among markers of oxidative stress and disease activity in SLE patients. (A) Relationship between ROS level and SLEDAI score. (B) Relationship between MDA level and SLEDAI score. (C) Relationship between GSH level and SLEDAI score. (D) Relationship between CD4 lymphocyte and SLEDAI score. (E) Relationship between total T lymphocyte apoptosis and SLEDAI score. (F) Relationship between $CD4^+$ T lymphocyte apoptosis and SLEDAI score.

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negative correlation existed between percentage of CD4 lymphocyte ($r = -0.522$, $p < 0.01$, Figure 1D) with disease activity in SLE patients. Furthermore, a significant positive correlation existed between disease activity with total T lymphocyte apoptosis ($r = 0.462$, $p < 0.01$, Figure 1E) and $CD4^+$ T lymphocyte apoptosis ($r = 0.543$, $p < 0.001$, Figure 1F) in SLE patients.

There were positive associations between intracellular ROS production with total T lymphocyte apoptosis ($r = 0.445$, $p < 0.01$, Figure 2A) and $CD4^+$ T cell apoptosis ($r = 0.460$, $p < 0.05$, Figure 2B) in SLE patients. Furthermore, the levels of lipid peroxidation was positively correlated with $CD4^+$ T cell apoptosis ($r = 0.620$, $p < 0.0001$, Figure 2C) in SLE patients. Interestingly, the levels of reduced glutathione were negatively associated with total T lymphocyte apoptosis ($r = -0.559$, $p < 0.001$, Figure 2D), $CD4^+$ T cell apoptosis levels ($r = -0.483$, $p < 0.01$, Figure 2E), $CD8^+$ T cell apoptosis ($r = -0.522$, $p < 0.01$, Figure 2F) and with intracellular caspase-3 activity in T lymphocytes ($r = -0.567$, $p < 0.001$, Figure 2G) and positively affiliated with percentage $CD4^+$ T cell ($r = 0.495$, $p < 0.01$, Figure 2H) in SLE patients. No such correlations were observed in healthy controls.

Discussion

Systemic lupus erythematosus is a chronic inflammatory autoimmune disease of multiple origins. The inflammatory nature of the disease infers that excessive ROS production and imbalance redox system may contribute to the immune dysfunction, autoantigen production and perturbation of programme cell death in SLE [5]. The phagocytic cells release ROS,

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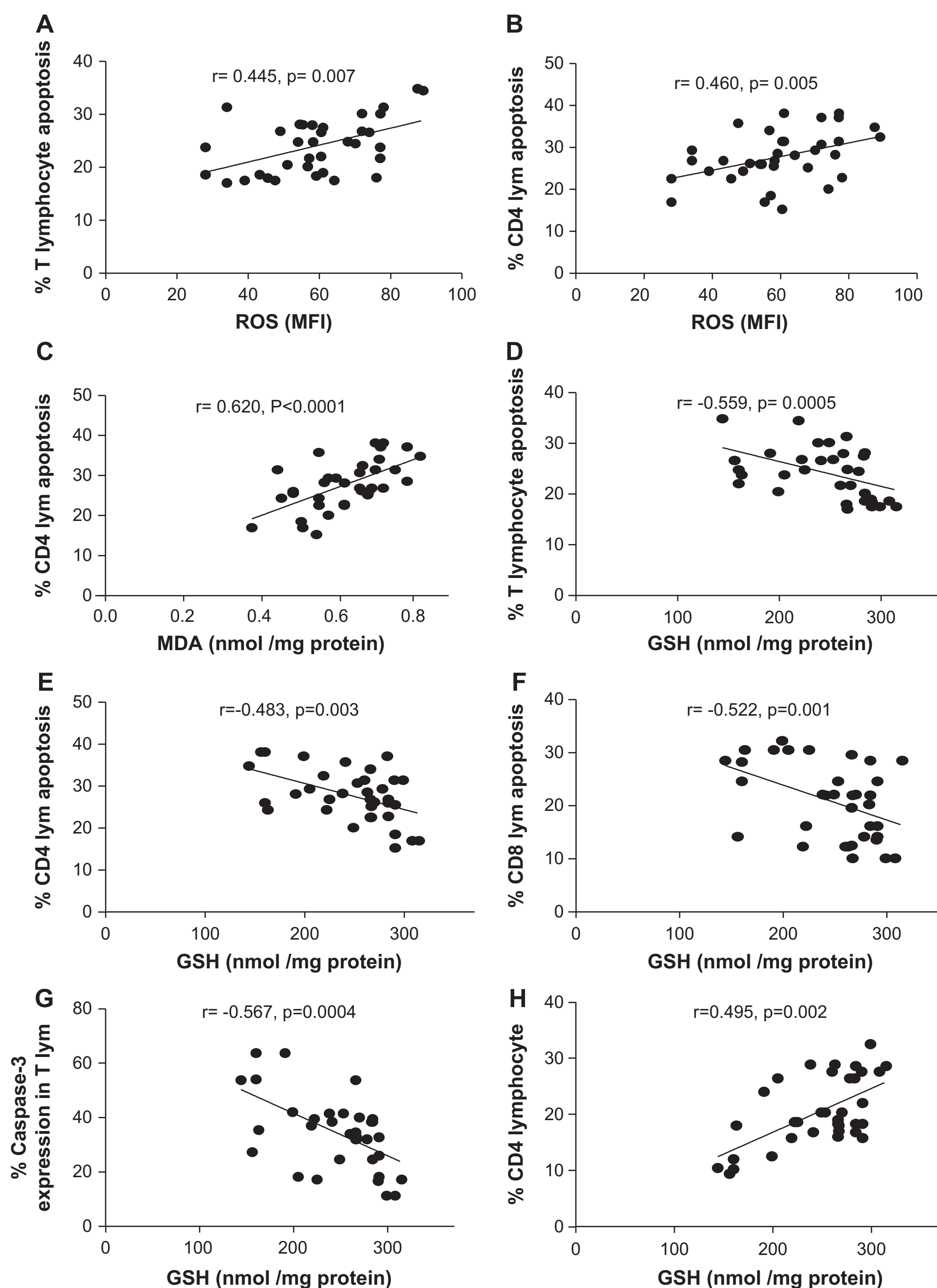

Figure 2. Correlation among T lymphocyte sub-sets, lymphocytes apoptosis and oxidative stress in SLE patients. (A) Relationship between ROS and total T lymphocyte apoptosis. (B) Relationship between ROS and CD4 lymphocyte apoptosis. (C) Relationship between lipid peroxidation and $CD4^+$ T cell apoptosis. (D) Relationship between GSH and percentage T lymphocyte apoptosis. (E) Relationship between GSH and $CD4^+$ T cell apoptosis levels. (F) Relationship between GSH and $CD8^+$ T cell apoptosis levels (G) Relationship between intracellular GSH levels and active caspase-3 expression in T lymphocytes. (H) Relationship between intracellular GSH levels and percent $CD4^+$ T cell.

which penetrate cellular membranes that react with nuclear/mitochondrial DNA, leading to perturbation of apoptosis [22]. The imbalance redox system delayed the clearance of apoptotic cells which may provide sustained interactions between ROS and apoptotic cell macromolecules generating neo-epitopes which subsequently result in autoantibody formation [7]. This further supports the already suspected role of oxidative stress in inflammatory disease. However, the liaison of oxidative stress and redox system in mononuclear cells with T lymphocyte sub-sets apoptosis in the severity of SLE remains to be elucidated. Thus, in the present study, we investigated the association between the oxidative stress, redox system and T lymphocyte sub-sets apoptosis in relation to disease severity in SLE. We found a strong connotation of oxidative

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stress and redox system with percentage T lymphocytes sub-sets and its apoptosis in SLE patients. The present study shows that excessive ROS production and decreased glutathione may be associated with deregulated lymphocyte sub-sets apoptosis, which together enhance the activity of disease.

Growing evidence suggests that intracellular ROS acts as a signalling molecule, involved in the maintenance of cellular redox (reduction/oxidation) homeostasis and defence mechanism in the immune system [23]; however, excessive ROS production led to the oxidative stress and may damage cell structures, including lipids, proteins and DNA molecules in SLE [24,25]. Oxidative damage mediated by ROS result in the generation of deleterious by-products, such as aldehydic products, leading to the formation of adducts with proteins that in turn make them highly immunogenic, thereby inducing pathogenic antibodies in SLE [26]. In the present study, a significantly increased level of intracellular ROS was observed in patients with SLE. If these reactive oxygen species are not scavenged, they lead to the lipid peroxidation. Increased levels of lipid peroxidation in the serum [3,27] and in the erythrocytes [28] were reported in the patients with SLE. In the present study, a significant elevated level of MDA (lipid peroxidation marker) was observed in the mononuclear cells from SLE patients. Increased level of lipid peroxidation indicated excessive production of intracellular ROS in SLE. The severity of disease measured as SLEDAI score was positively associated with increased levels of intracellular ROS production and lipid peroxidation in SLE, which was similar to the report of Tewthanom et al. [27]. The intracellular level of MDA production was intense in the nephritis patients. This result is similar to the study of Turi et al. [29] who showed that increased oxidative stress correlated with active glomerular disease in SLE patients. The positive correlations suggest that excessive production of ROS and lipid peroxidation may be involved in the enhancement of tissue damage in SLE patients and may reflect disease severity.

All the cell types, including lymphocytes and other immune system cells, have a complex machinery of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, thioredoxin, etc.) and antioxidant molecule (reduced glutathione, vitamins) for regulating oxidant reactions in the surrounding medium, thereby preventing free radical mediated cytotoxicity [30]. Adequate concentrations of glutathione are required for a variety of functions, including protection of the cell from oxidative damage by quenching of oxidant species, lymphocyte activation, natural killer cell activation and lymphocyte-mediated cytotoxicity [6,31]. In the present study, decrease in the levels of intracellular GSH showed a negative association with the severity of disease, especially with nephritis patients. Decreased intracellular GSH may be due to ROS-induced GSH oxidation or GSH export from cells; the resultant GSH reduction would enhance further ROS production during oxidative challenge [32]. In addition to the important role of GSH in the maintenance of oxidative system in cell, GSH oxidation is a major contributor to lymphocytes apoptosis mediated by oxidants [32].

Intracellular GSH depletion is an early hallmark in the progression of cell death in response to different apoptotic stimuli including activation of caspase and externalization of phosphatidylserine [6], thus custom a link between apoptosis and glutathione. Further, low doses of H_2O_2 found to induce apoptosis in varieties of cell types [33]; this observation also sustained the relation of oxidative stress and apoptosis. Apoptosis is a form of actively induced programmed cell death, with the characteristic features of chromatin condensation, DNA fragmentation and apoptotic body formation. Apoptotic bodies composed of numerous nucleolus bodies and organelles are normally removed by phagocytes as soon as they are formed. Failure to remove apoptotic bodies leads to the release of autoantigens which may induce autoimmunity [34]. Progressively studies demonstrated that lymphocytes apoptosis may play an important role in the pathogenesis of SLE [35]. In the present study, we found imbalance T lymphocytes sub-sets with increased $CD8^+$, decreased $CD4^+$ and a consequent reduction of $CD4^+/CD8^+$ T lymphocytes ratio in patients with SLE. The decrease levels of $CD4^+$ T lymphocytes were negatively associated with disease activity. Further, a significantly higher percentage of apoptotic and necrotic T lymphocytes were observed in SLE patients. Our reports are in accordance with a previous report by Jin et al. [36], who showed that percentage of apoptotic cells and necrotic cells were increased and were positively correlated with disease activity in SLE patients. Patients with decreased $CD4^+/CD8^+$ T lymphocyte ratio had arthritis, rash and increased serological activation. Seven of the patients with diminished $CD4^+/CD8^+$ T lymphocyte ratio had nephritis. These data may reflect the involvement of T lymphocytes apoptosis in the various clinical features of disease.

A remarkable increase activity of intracellular caspase-3 was found in T lymphocytes from SLE patients. The increased activity of intracellular caspase-3 was positively associated with severity of disease and negatively related to low intracellular glutathione levels, similar to the study of Xue et al. [37]. A negative association of the levels of GSH with T lymphocytes, $CD4^+$, $CD8^+$ lymphocytes sub-sets apoptosis and intracellular activated caspase-3 may support the role of reduced glutathione in the alteration of T lymphocytes apoptosis in the disease state. These results may suggest that glutathione has been involved in the depletion of $CD4^+$ T lymphocyte in SLE patients. is further supports an earlier study of Chang et al. [38] who showed that glutamine supplement enhances intracellular levels of glutathione and decreases T lymphocyte apoptosis.

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Furthermore, a strong positive correlation existed between apoptotic cell numbers and ROS production in SLE patients. Similarly, the increased numbers of apoptotic cells positively correlated with lipid peroxidation in SLE patients. These correlations suggest that GSH depletion has been shown to sensitize cells to agents that induce oxidative cell death, apoptosis, under these conditions has been assumed to be largely regulated by the signalling action of ROS. These data were supported by the study of Gergely et al. [39], who found that abnormal death signalling in lymphocytes of SLE patients had been associated with elevation of the mitochondrial transmembrane potential and increased production of reactive oxygen intermediates.

In conclusion, findings of this study imply that depletion of glutathione might be involved in the alteration of lymphocytes apoptosis. Also, analysis of redox state marker (GSH) with CD4/CD8 T lymphocytes may be helpful in monitoring the management of SLE. However, further longitudinal study needs to be taken up for evaluating the role of GSH as a therapeutic molecule on apoptosis and with severity of disease.

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Declaration of interest

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