

Correlation of Lipid Peroxidation and Glutathione Levels with Severity of Systemic Lupus Erythematosus: A Pilot Study from Single Center

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Received, December 11, 2007; Revised, May 29, 2008; Accepted, June 9, 2008; Published, June 18, 2008.

ABSTRACT – Purpose. Systemic Lupus Erythematosus (SLE) is a multifactorial chronic autoimmune disease with unidentified etiology. Imbalance of oxidative status is one possible cause of active disease. Plasma malondialdehyde (MDA) and plasma glutathione (GSH) level have been used as a determinate of oxidative status. Limited data has examined these 2 parameters by severity of SLE. **Methods.** We determined whether there was an association between plasma MDA and plasma GSH level with the severity of SLE. Forty four SLE patients (2 Men and 42 Women) and twenty healthy volunteers (3 Men, 17 women) participated in this study. SLE participants were classified by the severity of disease (mild, moderate or severe). The plasma MDA and plasma Glutathione levels were measured. The correlation of plasma MDA and plasma GSH levels with the severity of SLE disease were determined. **Results.** Plasma MDA levels with different severity of SLE (mild, moderate, and severe of SLE patients) were not significantly different from those of the control group ($p=1.0$). Plasma GSH levels were significantly lower in the moderate and severe SLE groups than the control group ($p=0.001$). In addition, a significant correlation between plasma GSH and severity of SLE was observed. (Pearson correlation coefficient = -0.428 , $p < 0.001$). The relationship could be described by the equation $GSH \text{ level } (\mu M) = (-7.624) \text{ SLEDAI score} + 545.90$. **Conclusion.** A significant correlation between plasma GSH and SLE severity exists that may aid evaluation of the disease severity and usefulness of the treatment of SLE.

INTRODUCTION

Systemic Lupus Erythematosus (SLE) is a complex chronic immunological disorders with unclear etiologies. There has been attempted to investigate the exactly cause of this disease and many possible theories have been proposed. One of the possible theories about pathology of this disease is an imbalance of the oxidative status of SLE patients. Many researches in animals and in human showed the increasing of lipid peroxidation and decreasing of Glutathione level in animals and patients with this disease [1-10]. However, no research has concentrated on the correlation of lipid peroxidation and Glutathione (GSH) level with different severity of this disease has been presented. Therefore, the aim of this study was to investigate the correlation between lipid peroxidation and the circulating concentrations of GSH and malondialdehyde (MDA), a lipid peroxidation product, with the degree of severity of SLE which may further implied for better understanding of the pathology of this disease.

METHODS

Patients

This study was performed at the Division of Allergy, Immunology and Rheumatology of Ramathibody Hospital, Bangkok. All active SLE patients who came to the division during March 2006 to July 2006 and agreed to be included into the project were recruited and classified into 3 groups by severity based on the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [11, 12]: Mild, score < 10 ; Moderate, score $10-20$; Severe, score > 20 .

The classification of severity was made by physicians. Some patients in Moderate and Severe SLE groups who agreed to participate in

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the project were recruited from inpatient department (IPD).

Twenty healthy volunteers with no underlying diseases were also recruited to be a control group of the study.

The project had been approved by the Ethic Committee of the hospital and written informed consents were obtained from all participants.

Blood samples collection and biochemical measurement

Five ml of blood samples were drawn and immediately chilled. Plasma was separated by centrifugation at 2° C at 3000 rpm for 10 min. The specimens were kept at -20° C and analyzed within 2 weeks.

GSH analysis was performed using a commercially available assay kit (Bioassay Inc, CA, USA). MDA was measured according to the method of Yagi [13], using microplate reader (Victor2 Perkin Elmer Inc, Turku, Finland) as a detector.

Statistical Analysis

SPSS version 11.5 software (SPSS Inc, Chicago, IL) was used to perform analysis of variance (ANOVA) followed by Bonferoni's test as the post hoc test to identify which mean was different from others, correlation analysis. The Pearson correlation was performed to determine the association of MDA, Glutathione level and the severity of SLE patients. The statistical significant difference was considered when p-value was less than 0.05.

RESULTS

In 5 months, 44 SLE patients (2 men and 42 women) were recruited into the study and were classified into the preset three groups; Mild (20 women), Moderate (15 women), and Severe (2 men and 7 women) (Table 1). The age, smoking habits and SLE duration were not significant difference between groups including Control group (3 Men, 17 women). There was no significant difference in prednisolone and antimalarial drugs use between the patients group. The use of other immunosuppressives, however, was lower in Mild as compared with other two groups. In addition, prednisolone dose (mg/week) was larger (mean \pm SEM) in more severe SLE cases (mild = 86.9 \pm 110.0, moderate = 145.83 \pm

79.8, severe = 337.5 \pm 187.8). There was significant difference in chloroquine doses between Mild and Moderate groups and also between Moderate and Severe groups. Similarly, the dose of cyclophosphamide was significantly higher in the moderate cases as compared with others.

All of the control volunteers were healthy as determined by physicians having normal diet and agility with no underlying diseases such as cardiovascular or allergic disorders that may affect the oxidative status.

GSH and MDA concentrations in Plasma

GSH. As depicted in Figure 1 and Table 2, there were significant difference in the mean and 95% confidence interval (CI) value of plasma GSH concentrations of patients with different severity of SLE. The values were significantly higher in Moderate and Severe as compared with Control. There was no significant difference between Mild and Control.

MDA. The mean and 95% CI were not significantly different among the four groups (Figure 2, Table 2).

Correlation of Plasma MDA and GSH with Severity of SLE

There was a significant negative correlation between plasma GSH and severity of SLE (Pearson correlation coefficient = -0.479, p=0.001). The regression equation to predict the GSH level based on the severity of SLE disease was, GSH level (μ M) = -132.25 Group severity + 655.578 (p= 0.001). Where dummy or indicator variables had been used for group severity 1 for Mild, 2 for Moderate and , 3 for Severe. These numbers do not represent any actual measurements; they simply identify the categories of the nominal random variable.

There was a positive but not significant trend for correlation between plasma MDA concentration and the SLE severity (Pearson correlation coefficient = 0.166, p=0.281).

In addition, the correlation between scores oxidative status parameters and SLE severity were re-analyzed using the continuous SLEDAI scores in place of the categorized group severity scale. Nearly the same results were observed.

Significant correlation between SLEDAI and GSH levels was observed (Pearson correlation coefficient = -0.428, $p < 0.001$) with the following equation: GSH level (μM) = -7.624 SLEDAI + 545.90.

MDA levels were showed a trend for relations with SLEDAI score, however, the correlation did not reach significance (Pearson correlation coefficient = 0.174, $p = 0.084$).

Table 1. The demographic data of subjects ^a

	Control (n=20)	SLE patients			p-value
		Mild (n=20)	Moderate (n=15)	Severe (n=9)	
Age, years	44.9 ± 15.6	37.7 ± 13.2	36.3 ± 11.5	39.8 ± 13.6	0.573
Smoking	No	No	No	No	1.00
SLE duration, years	0	4.5 ± 3.3	5.73 ± 6.3	6.6 ± 3.9	1.00
SLEDAI score	0	3.4 ± 1.8	13.3 ± 1.3	37.8 ± 8.7	0.0001*
Drugs being used:					
Prednisolone (%)	0	85	100	100	0.151
Prednisolone dose, mg/wk	0	86.9 ± 110.0	146 ± 80	338 ± 188	0.0001*
Antimalarial (%)	0	65	87	67	0.335
CQ dose, mg/wk	0	400 ± 676 ^b	1183 ± 765 ^{b,c}	389 ± 772 ^c	0.0001*
HCQ dose, mg/wk	0	410 ± 614	160 ± 429	622 ± 738	0.007*
Other Immunosuppressives (%)	0	25	67	56	0.042
AZA dose, mg/wk	0	35.0 ± 107.7	140 ± 178	117 ± 175	0.006*
CYP dose, mg/wk	0	35.0 ± 108 ^d	227 ± 350 ^d	77.8 ± 154	0.007*

^a all values are expressed as mean ± SD; ^b $p = 0.002$ (mild vs moderate); ^c $p = 0.016$ (moderate vs severe); ^d $p = 0.025$ (mild vs moderate); * significantly different at $p < 0.05$; CQ = chloroquine; HCQ = hydroxychloroquine; AZA = azathioprine; CYP = cyclophosphamide.

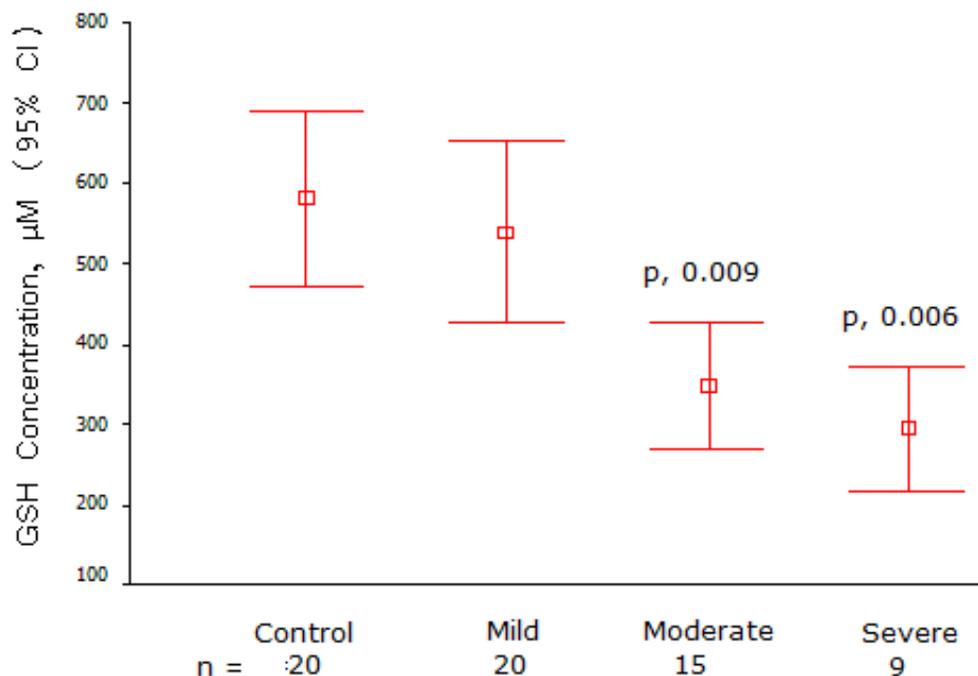


Figure 1. The mean and 95% CI of plasma GSH concentrations (μM) of patients with different severity of SLE ($p < 0.05$, significantly different from Control)

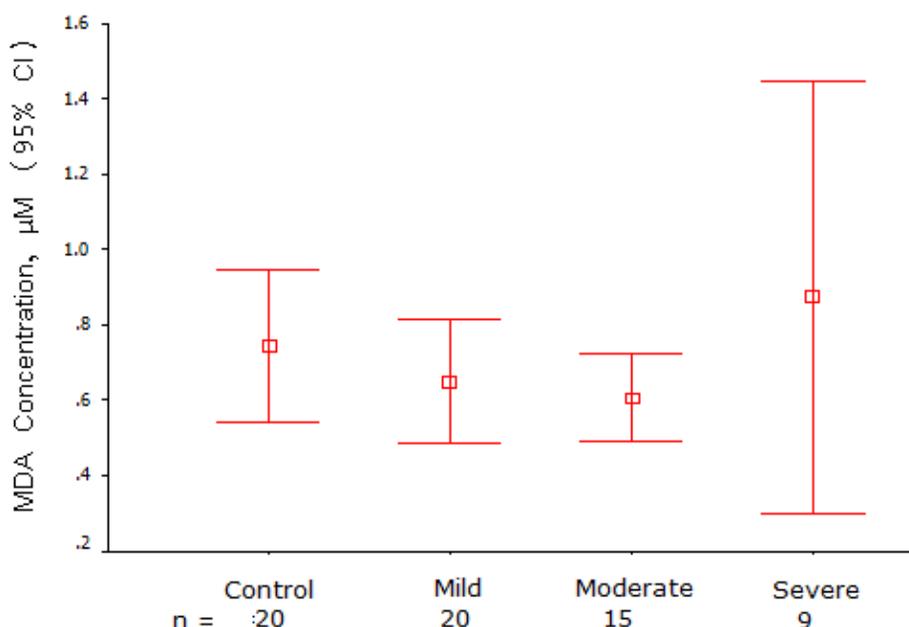


Figure 2. The mean and 95% CI of Plasma MDA concentration (μM) of patients with different severity of SLE.

Table 2. Mean and SEM of oxidative status parameters in SLE and control patients

	Control (N=20)	Mild SLE (N=20)	Group Moderate SLE (N=15)	Severe SLE (N=9)
Glutathione, μM	583 \pm 52	540 \pm 54	348 \pm 37	295 \pm 34
p value against Control		0.915	0.009	0.006
Malondialdehyde, μM	0.74 \pm 0.097	0.65 \pm 0.078	0.61 \pm 0.055	0.88 \pm 0.249
p value against Control		1.00	1.00	1.00

DISCUSSION

Base on the evidence that imbalance of oxidative status is associated with the pathology of SLE [1, 5, 10, 14, 15], this study was performed to determine the correlation of oxidative status parameters and the degree of severity of SLE disease. A significant correlation was observed between plasma GSH and SLE severity. However, our work had several limitations. First, the blood sample from every patient was not collected at the same time of the day and this was a single point measurement. Second, there were

several other factors which might have influence on GSH levels, for example, duration of SLE, and the use of prednisolone, antimalarial, and other immunosuppressive medications. The duration of SLE, however, may be ruled out as there was no significant difference between groups with this regard (Table 1). The use of prednisolone, antimalarials and other immunosuppressive medications as confounding factors might also be ruled out as, their use is expected to increase and not, as we observed, decrease GSH concentrations.

For MDA levels, the relationship between MDA levels and SLEDAI score was less obvious

than that reported previously [6]. This may be due to the effect of prednisolone on lipid peroxidation [16] and/or other immunosuppressive medications, since the recruited patients in the previous report were studied before the starting of SLE treatment. That implies that the treatment may result in normalization of MDA levels by improving the oxidative status.

In a recent study of a relationship of oxidative status and SLE severity, a different oxidative stress parameter, i.e., isoprostane has been monitored that is related to the fatigue symptom [17]. Therefore, this study is the first to report the relationship between glutathione level and SLE severity.

In conclusion, a significant correlation between plasma GSH and SLE severity exists that may aid evaluation of the disease severity and usefulness of the treatment of SLE.

ACKNOWLEDGEMENT

The authors are grateful to Chulalongkorn University for financial support. Special grateful acknowledgement was regarded to all health care members (nurses, residents, medical staffs, officers) at Ramathibodi hospital who provided a kind assistance during the study.

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