

## ORIGINAL ARTICLE

# ELEVATED LIPOPROTEIN(a) IN SYSTEMIC LUPUS ERYTHEMATOSUS

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**Background** – The underlying mechanism(s) for the development of premature atherosclerosis in patients with systemic lupus erythematosus (SLE) is not precisely understood. In recent years, lipoprotein(a) [Lp(a)] has emerged as a valuable predictor as well as an independent risk factor for premature atherosclerosis. The objective of this study was to determine blood Lp(a) levels in SLE patients, and to determine whether increased levels are a contributing factor to the development of atherosclerotic complications of SLE.

**Methods** – Serum Lp(a) levels were measured by enzyme immunoassay in 37 SLE patients, nine of their apparently healthy siblings and 35 healthy controls. Student's *t* test was used for statistical comparisons.

**Results** – Our data showed a higher frequency of enhanced Lp(a) levels (> 30 mg/dL) in SLE patients as compared with that observed in healthy controls (51.4% vs 25.7%). Analysis of lipid profiles in SLE patients also revealed significantly higher levels of triglycerides as compared with controls ( $165 \pm 16$  vs  $100 \pm 6$  mg/dL,  $p = 0.001$ ). Serum Lp(a) levels were not different between patients with or without glucocorticoids, hospitalization and disease exacerbation ( $p$  values equal to 0.9, 0.7 and 0.6, respectively). Moreover, there was no significant difference in serum Lp(a) concentration between nine SLE patients and their siblings ( $34 \pm 11.8$  vs  $37.3 \pm 12.8$  mg/dL;  $p = 0.8$ ).

**Conclusion** – Increased Lp(a) levels are more likely to be encountered in patients with SLE than in healthy subjects. Lp(a) blood levels in SLE, as in healthy individuals, seem to represent a genetically predetermined trait.

**Keywords** • atherosclerosis • lipoproteins • lupus erythematosus

## Introduction

Patients with systemic lupus erythematosus (SLE) exhibit an increased incidence of atherosclerotic and thromboembolic complications.<sup>1</sup> Atherosclerosis has been acknowledged as a major cause of death and morbidity in SLE patients.<sup>2,3</sup> The reason for development of premature atherosclerosis in SLE is not precisely known, but the higher prevalence of atherosclerotic progression may be ascribed to

numerous factors including primary immunologic injury to blood vessels, presence of anti-phospholipid antibodies, hypertension, vasculitis, duration of glucocorticoid therapy, and dyslipoproteinemia.<sup>2,4,5</sup> Arteriovenous thrombosis is also a well-known complication of SLE.<sup>6</sup>

In recent years, there has been a considerable resurgence of interest in lipoprotein(a) [Lp(a)], as there is strong evidence that it represents an independent risk factor for premature atherosclerosis. Lp(a) is composed of a low density lipoprotein (LDL) linked by disulfide bonds to apolipoprotein(a) [apo(a)], a high-molecular weight protein with striking homology to a serine

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protease, plasminogen. The LDL-like property of Lp(a) confers the particle an atherogenic potential, while the similarity of the apo(a) subunit to plasminogen could account for prothrombotic characteristic of Lp(a).<sup>7,8</sup>

Current information justifies the need to determine plasma Lp(a) levels in patients who are susceptible to atherosclerotic cardiovascular disease.<sup>9</sup> On the other hand, it has been suggested that Lp(a) may be involved in immunologic mechanisms.<sup>10</sup> Therefore, it is important to compare Lp(a) levels in SLE patients with those in healthy subjects. In addition, serum Lp(a) levels should be determined in these patients' healthy siblings to find out whether or not there is a genetic tendency towards higher Lp(a) levels in lupus patients.

## Patients and Methods

### Subjects

Thirty-seven SLE patients with a mean age of 28 years (range, 13 – 53) including 32 women and 5 men constituted the patient group. All patients had exhibited at least four of the revised criteria for the diagnosis of SLE<sup>11</sup> during the course of their disease. All had been admitted to the rheumatology centers of the three main medical universities of Tehran between April 1994 and October 1995, although they were from different parts of Iran. Two sisters with inactive SLE were among the patients. Twenty-nine patients were taking methylprednisolone and eight were not. None of them was receiving lipid-lowering drugs. Sixteen patients had been hospitalized in rheumatology wards because of an SLE flare-up, while 21 were seen during outpatient follow-up periods in the Rheumatology Clinic of Loghman Hakim General Hospital. Within our outpatient group, 20 had inactive SLE and only one had active disease according to the lupus activity criteria count described by Urowitz et al.<sup>12</sup> None of the patients had overlap syndromes. In this study, patients with nephrotic syndrome, diabetes mellitus, hyper- or hypothyroidism were not included because of reported changes in plasma Lp(a) levels related or secondary to these conditions.<sup>13-15</sup> A pregnant woman and a patient with Budd-Chiari syndrome were also excluded from this study because of the fluctuations of plasma Lp(a) levels during pregnancy<sup>16</sup> and the fact that the liver is involved in the synthesis of Lp(a),<sup>17</sup> respectively. In addition, those patients who had undergone hemodialysis or recent surgical operations were not

included because Lp(a) levels may change under these circumstances.<sup>16,17</sup>

The control group consisted of 35 healthy volunteers who had been referred to the Iranian Reference Laboratory in order to undergo routine tests before marriage (opium test and Venereal Disease Research Laboratory test). An appropriate questionnaire was used to ensure that the control group included only healthy subjects. Despite the fact that Lp(a) concentration is not correlated with age or sex,<sup>18</sup> controls and patients were matched for both age and sex. Because Lp(a) blood level is known to be transmitted as an autosomal dominant trait,<sup>19</sup> Lp(a) blood levels were determined in our patients' healthy siblings. After a detailed clinical examination, nine apparently healthy siblings (one for each patient) were selected, in whom there was no sign or symptom of clinical lupus.

### Laboratory investigations

Blood specimens were obtained after a 12-hour fast. Serum Lp(a) was determined by sandwich enzyme-linked immunosorbent assay (ELISA) using an Immunozymp Lp(a) kit provided as a gift by IMMUNO AG (Vienna, Austria). All known isoforms of Lp(a) were detectable by the anti-apo(a) polyclonal antibodies employed in this kit. Furthermore, according to the manufacturer, cross-reactivity of Lp(a) with plasminogen or LDL does not interfere with the test results. Total serum cholesterol, triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic colorimetric methods using Technicon kits purchased from MAN Laboratories (Tehran, Iran). HDL-C was determined after precipitating very-low-density lipoprotein (VLDL) and LDL with a reagent containing dextran sulfate and magnesium chloride. VLDL-cholesterol (VLDL-C) was calculated by dividing the TG concentration by 5, and LDL-C was calculated by the Friedwald formula,<sup>20</sup> where  $LDL-C = total\ cholesterol\ (TC) - [HDL-C + (TG/5)]$ .

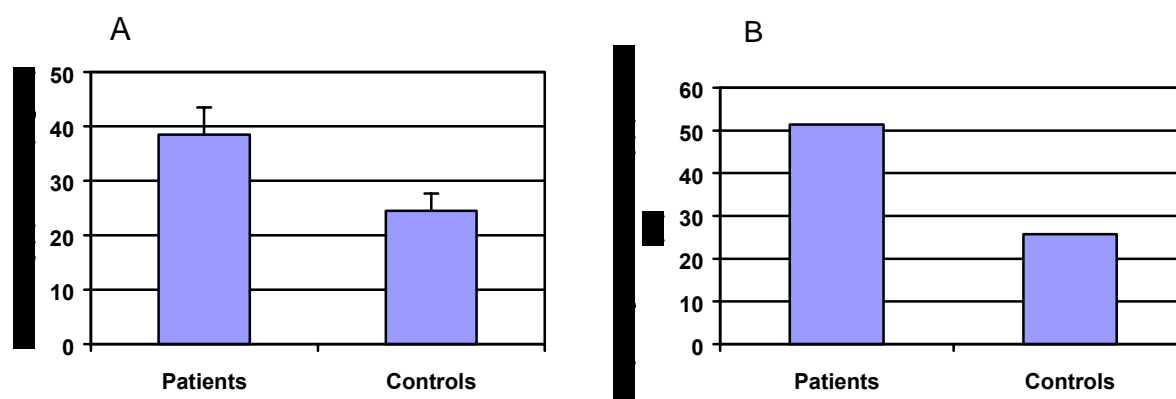
### Statistical analysis

Data were compared using Student's *t* test and *p* values less than 0.05 were considered statistically significant. All analyses were performed using SPSS/PC+ software (version 6.1). The results are expressed as mean ± standard error (SE).

## Results

There was a strong trend toward abnormally

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**Figure 1.** Lipoprotein(a) [Lp(a)] concentration in SLE patients and controls. A) There was a trend toward higher Lp(a) blood levels in SLE patients as compared with controls ( $38.5 \pm 6.4$  vs  $24.5 \pm 4.3$  mg/dL,  $p = 0.07$ ). B) Elevated Lp(a) levels ( $> 30$  mg/dL) were also more frequent in SLE patients (51.4% vs 25.7%).

Lp(a) concentrations in SLE patients as compared with controls, although the observed difference did not reach statistical significance ( $38.5 \pm 6.4$  vs  $24.4 \pm 4.3$  mg/dL;  $p = 0.07$ ) (Figure 1A). However, the frequency of high Lp(a) levels ( $> 30$  mg/dL) was twice as high in patients with SLE than in healthy subjects (Figure 1B).

There was no difference in mean serum Lp(a) levels between patients with and without glucocorticoid therapy ( $p = 0.9$ ), hospitalized and nonhospitalized patients ( $p = 0.7$ ), and those with and without active disease ( $p = 0.6$ ) (Table 1). We found raised serum levels of TG and VLDL-C in SLE patients as compared with controls ( $165 \pm 16$  vs  $100 \pm 6$  mg/dL and  $33 \pm 3$  vs  $20 \pm 1$  mg/dL, respectively;  $p = 0.001$ ), which is consistent with the so-called “lupus pattern” of dyslipoproteinemia.

Furthermore, the statistical correlation analyses of the data revealed no significant differences between patients on or not on glucocorticoid therapy in terms of serum levels of TC, TG, HDL-C, LDL-C and VLDL-C.

To explore a possible genetic basis for high blood Lp(a) levels in SLE patients, serum Lp(a) concentrations of nine SLE patients were directly compared with those of nine of their apparently

healthy siblings. No difference in mean Lp(a) levels was noted between these two groups ( $34 \pm 11.8$  mg/dL vs  $37.3 \pm 12.8$  mg/dL;  $p = 0.8$ ). Furthermore, two sisters with inactive SLE had quite similar serum Lp(a) concentrations (92.2 mg/dL and 88.3 mg/dL).

## Discussion

Atherosclerotic and thromboembolic problems are among the life-threatening, long-term complications of SLE. Dyslipoproteinemia is widely recognized as a risk factor for vascular disease in lupus.<sup>5,21</sup> Serum samples from SLE patients have been shown to stimulate the accumulation of cholesterol in cultured smooth muscle cells of the aorta. Such an atherogenic effect of lupus sera was attributed to the presence of LDL-containing immune complexes.<sup>22</sup> McGregor et al reported SLE cases with elevated apolipoprotein B levels in spite of normal blood levels of cholesterol and LDL-C. They suggested that SLE patients may have abnormally dense particles of LDL.<sup>1</sup>

The LDL-like molecule Lp(a) has become a focus of attention due to the possibility that levels

**Table 1.** Serum lipoprotein(a) levels (mg/dL) of SLE patients in relation to glucocorticoid therapy, hospitalization and disease activity.

Variable	Yes (n, F/M)	No (n, F/M)	p Value
MP therapy	$38.1 \pm 2.7$ (29, 25/4)	$39.9 \pm 14.9$ (8, 7/1)	0.9
Hospitalization	$35.8 \pm 10.7$ (16, 14/2)	$40.6 \pm 8$ (21, 18/3)	0.7
Active disease	$34.9 \pm 10.2$ (17, 15/2)	$41.6 \pm 8.3$ (20, 17/3)	0.6

*n* = number of subjects; F = female; M = male; MP = methylprednisolone.

circulating Lp(a) represent an independent risk factor for coronary vascular disease of greater predictive potential than other lipoprotein traits.<sup>23</sup> Elevated Lp(a) levels in lupus patients have been previously reported.<sup>6,24</sup> Lp(a) blood levels in our study patients tended to be higher than in controls, although such a difference did not reach statistical significance possibly because of the relatively small number of patients examined. Alternatively, it may reflect genetic or population-based differences between the patients in this study and those in earlier similar studies, as Lp(a) levels are known to vary widely among various ethnic groups.<sup>25</sup> The latter possibility is supported by a recent study conducted in Tehran, in which Lp(a) serum levels were not different between patients with angina pectoris and healthy controls.<sup>26</sup> Nevertheless, our results clearly indicate a higher frequency of increased Lp(a) levels (> 30 mg/dL) in SLE patients. The clinical importance of Lp(a) in SLE are emphasized by the results of a retrospective study in which there were higher levels of Lp(a) in eight SLE patients with myocardial and/or cerebral infarction than in eight SLE patients with no history of infarction.<sup>27</sup>

Lp(a) concentrations are not affected by most pharmacologic agents. However, the general consensus is that the identification of elevated Lp(a) levels in a patient should prompt the clinician to search for and eliminate modifiable risk factors such as cigarette smoking, arterial hypertension, diabetes mellitus and hypercholesterolemia.<sup>28,29</sup> Furthermore, because the cardio-vascular risk of Lp(a) increases when plasma levels of LDL-C are also high,<sup>28,30</sup> LDL-C concentrations can be lowered therapeutically at the appropriate time.

The effect of glucocorticoid therapy on blood Lp(a) levels has been an issue of controversy. In this study, there was no difference in Lp(a) concentration between patients with and without glucocorticoid therapy, nor was there any significant correlation between Lp(a) levels and methylprednisolone dosage. This is in agreement with the results of Matsuda et al<sup>6</sup> and Borba et al,<sup>24</sup> but not with those of another report wherein reductions in serum Lp(a) levels following glucocorticoid therapy was found in a small group of patients.<sup>31</sup> Further studies on greater numbers of lupus patients are required to clarify whether or not glucocorticoid therapy can actually alter Lp(a) blood levels.

Our finding that Lp(a) levels did not differ

between those patients with and those without active disease lend support to the results of Borba et al,<sup>24</sup> indicating that the acute phase reactant-like property of Lp(a)<sup>16</sup> is unlikely to be responsible for raised Lp(a) levels encountered in SLE patients.

Plasma Lp(a) levels are known to be genetically determined, as supported by familial and twin studies.<sup>9</sup> However, no attempts to correlate Lp(a) blood levels of SLE patients with those of their healthy siblings have ever been made. Our results extend the existing literature in that Lp(a) blood levels are not different between lupus patients and their healthy siblings. In fact, in our examination of lupus patients and their healthy siblings, 50% of the pairs showed almost identical Lp(a) levels. Besides, two sisters (aged 34 and 39) with inactive SLE, taking the same daily dosage of methylprednisolone (2.5 mg/day), had very similar serum Lp(a) concentrations (92.2 and 88.3 mg/dL, respectively). Thus, it could be concluded that the higher frequency of elevated Lp(a) levels in lupus patients as compared with healthy subjects represents a phenotypic expression of a related genetic trait. This is by no means a cause and effect relationship. Rather, these data may provide a clue to a genetic tendency toward higher Lp(a) levels in patients with SLE. More detailed and controlled investigations on greater numbers of cases are warranted. The ever-enigmatic physiologic role and other functional aspects of Lp(a), as well as its modifications manifested in other autoimmune conditions, also remain to be elucidated.

## Acknowledgment

*The authors wish to thank Dr. Earl D. Silverman of the University of Toronto for critical review of this manuscript. This study was made possible by a generous gift of an Immunozygm Lp(a) kit from IMMUNO AG, Vienna, Austria.*

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