



Serum interleukin-18 and its relationship with subclinical atherosclerosis in systemic lupus erythematosus

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Original Article

Abstract

BACKGROUND: Interleukin-18 (IL-18) is a pro-inflammatory and pro-atherogenic factor, and its blood level has shown a direct correlation with atherosclerosis. We aimed to evaluate the serum IL-18 level in patients with systemic lupus erythematosus (SLE) and its relationship with the intima-media thickness (IMT) of the carotid artery in these patients, as an indicator of atherosclerosis.

METHODS: In this cross-sectional study, 60 patients as the patient group and 30 healthy volunteers as the control group [matched sex, age, and body mass index (BMI)] were selected, and their disease status and general data were gathered using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) form. A blood sample was also obtained from all participants to determine the serum level of IL-18 and other metrics, including high-sensitivity C-reactive protein (hs-CRP), cholesterol, triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), anti-double stranded deoxyribonucleic acid (anti-dsDNA), complement 3 (C3), and C4. The IMT of the carotid artery was calculated in both groups. We also evaluated the clinical cardiovascular manifestations.

RESULTS: The serum IL-18 levels in patients were significantly higher than in the control group ($P < 0.005$). It had no significant correlation with disease activity ($P = 0.10$). The patients with SLE with high IL-18 serum levels (> 280 pg/ml) had higher SLEDAI-2K ($P = 0.02$) than the patients with a low level (< 280), where 280 was the median of the IL-18 levels. The serum IL-18 level had no significant correlation with the carotid artery IMT.

CONCLUSION: A high level of IL-18 reflects the disease activity, but it was not significantly correlated with subclinical atherosclerosis, denoted by the carotid artery IMT.

Keywords: Systemic Lupus Erythematosus; Interleukin-18; Atherosclerosis

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Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of unknown etiology,¹ with diverse manifestations and multi-organ involvement.²⁻⁴ The overproduction of proinflammatory cytokines has been recognized as the main feature of SLE.¹ Interleukin-18 (IL-18) is an important inflammatory cytokine and a pro-

inflammatory and pro-atherogenic factor. Its serum

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level has a direct correlation with atherosclerosis in the coronary artery.⁵ We aimed to evaluate the relationship between the serum IL-18 level and the disease activity, with the carotid artery intima-media thickness (IMT) as a marker of atherosclerosis in patients with SLE.⁶

Materials and Methods

This cross-sectional study was conducted in Ghaem Hospital of Mashhad, Iran, from February 2013 to September 2016, on 60 patients with SLE of 20 to 60 years old, with at least 5 months of disease history who fulfilled the Systemic Lupus Collaborating Clinics (SLICC) criteria 2012.⁷ The control group consisted of 30 healthy volunteers, with matched age, sex, and body mass index (BMI). Both groups completed Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) forms,⁸ after obtaining informed consent. Further, their medical history and different laboratory features through blood and morning urine samples were gathered in a single-blinded manner. Patients with other rheumatologic or non-rheumatologic inflammatory disorders, pregnant women, and very ill patients were excluded.

Blood samples, after separating the serum, were kept frozen at -70 °C. An IL-18 BMS267/2 enzyme-linked immunosorbent assay (ELISA) Research Kit (Bender MED) was used to determine the serum level of IL-18. All subjects underwent a B-mode duplex carotid Doppler ultrasonography (Medison 8000EX) with a linear 10 MHz probe to determine the IMT of the carotid artery. Atherosclerotic plaques were identified as a specific area with hyper-echogenicity or local elevations into the lumen of the artery, involving at least 50% of the artery circumference.

Statistical analysis: To present normally-distributed variables, mean \pm standard deviation (SD) and for other quantitative variables, median and interquartile range (IQR) were used. We used the Kolmogorov-Smirnov test to identify if the samples followed a normal distribution pattern or not. In case that a sample did not follow a normal distribution pattern, we used non-parametrical tests including Mann-Whitney and Kruskal-Wallis tests. We used independent samples t-test for data following a normal distribution pattern. A P-value of < 0.05 was considered statistically significant. Data were analyzed using SPSS software (version 22.0, IBM Corporation, Armonk, NY, USA). The median of the IL-18 levels, equal to 280, was selected as the cut-off point.

Results

The female to male ratio was 55/5 in patients and

26/4 in controls ($P = 0.474$). The mean duration of SLE in patients was 57.80 ± 60.91 months (median: 36, IQR: 54, $Q1 = 18.0$, $Q3 = 72.0$), and the mean corticosteroid consumption duration was 49.80 ± 51.70 months. Descriptive statistics are provided in table 1.

The major organ involvement in the patient group was the kidney (31.7%), followed by the heart (18.3%), with 1.7% cardiovascular intervention, 5.1% angina pectoris, and 11% pericarditis, and the central nervous system (CNS) (8.3%), including psychosis (3.3%), seizure (3.3%), and cerebrovascular accident (CVA) (1.7% of cases).

Regarding the qualitative variables in the patients' group, they had a history of smoking (1.7%), diabetes (3.4%), angina pectoris (5.1%), cardiovascular intervention or CVA (1.7%), peripheral artery conditions (10.2%), coronary artery disease (CAD) in their 1st-degree relatives (5%), and CNS complications (8.3%), and no patient had a history of intermittent claudication. Further, in 78.3% of patients, the blood pressure was less than 140/90 mmHg, 26.7% of patients had a history of antihypertensive medication usage, only 15% had a history of premature ovarian failure (POF), and 18.3% reported a history of cardiovascular complications. Antinuclear antibody (ANA) was positive in 83.3% of the patients. All indicators were absent in the control group, except the CAD history in the 1st-degree relatives, which was 4.9%.

There was a significant difference in the history of taking antihypertensive medications ($P = 0.04$). There was also a significant difference in serum levels of IL-18 between patients and controls ($P < 0.005$). The mean of constraint-induced movement therapy (CIMT) was 0.396 ± 0.078 and 0.362 ± 0.080 for the patient and control groups, respectively ($t = 1.92$, $P = 0.057$).

Comparing SLEDAI between the two groups, only one significant difference was found, showing a higher disease activity in subjects with higher serum levels of IL-18 ($P = 0.02$). There was no significant correlation between the IL-18 serum level and the IMT of the carotid artery.

Regarding the patient group, there was no significant relationship ($P = 0.133$) between the serum level of IL-18 and the severity of the disease, dividing patients based on their SLEDAI score into three groups: < 5 ($n = 30$): 257.60 ± 54.01 pg/ml, $5-10$ ($n = 13$): 427.46 ± 89.41 , and > 10 ($n = 17$): 407.71 ± 71.99 . No significant difference was found either between the IL-18 serum level and cardiovascular risk factors (Table 2).

Table 1. Comparison of quantitative variables in patients and controls (if present), and two groups of patients by the serum interleukin-18 (IL-18) level (if applicable)

Characteristics	Patients vs. controls (if applicable)			Comparison by serum IL-18 levels (patients)		
	Patient group	Control group	P	< 280 pg/ml	≥ 280 pg/ml	P
	(n = 60)	(n = 30)		(n = 30)	(n = 30)	
Age (year)	28.80 ± 10.30	33.80 ± 9.10	0.781	28.53 ± 1.77	29.11 ± 2.06	0.832
BMI (kg/m ²)	23.90 ± 4.70	24.40 ± 5.10	0.719	23.80 ± 0.73	24.11 ± 1.18	0.815
IMT (total) (mm)	0.39 ± 0.07	0.36 ± 0.08	0.057	0.37 ± 0.06	0.39 ± 0.09	0.198
hs-CRP (mg/l)	0.93 (0.30-3.60)	0.60 (0.31-2.50)	0.164	2.66 ± 0.56	2.24 ± 0.57	0.603
Cholesterol (mg/dl)	182.00 ± 43.00	154.00 ± 50.00	0.002	174.50 ± 6.83	189.82 ± 8.87	0.171
TG (mg/dl)	135.00 ± 46.00	74.00 ± 38.00	< 0.001	129.25 ± 8.02	141.96 ± 8.62	0.284
LDL (mg/dl)	121.00 ± 32.00	95.00 ± 43.00	< 0.001	117.13 ± 5.40	125.14 ± 6.50	0.346
HDL (mg/dl)	45.00 ± 9.00	43.00 ± 5.00	0.867	45.40 ± 7.80	42.10 ± 8.20	0.072
IL-18 (pg/ml)	336.93 ± 39.69	96.89 ± 16.15	< 0.001	99.60 ± 70.60	590.80 ± 264.00	< 0.001
SLEDAI [*]	4.50 (2.00-12.00)	-	-	5.72 ± 1.14	9.93 ± 1.44	0.024
Anti-dsDNA [*] (IU/ml)	107.00 (64.50-296.50)	-	-	234.03 ± 46.98	206.75 ± 39.17	0.663
C3 (mg/dl) [*]	48.00 (24.50-88.00)	-	-	71.09 ± 8.96	58.10 ± 8.58	0.303
C4 (mg/dl) [*]	22.00 (12.00-35.75)	-	-	31.09 ± 5.92	26.48 ± 3.24	0.514
FBS (mg/dl)	84.40 ± 18.10	74.20 ± 18.30	0.014	80.41 ± 2.01	89.11 ± 4.36	0.064
Lymphocyte count [*]	5715.00 ± 2543.00	-	-	5683.00 ± 319.00	5753.00 ± 609.00	0.916
Platelet count [*]	231266.60 ± 85875.30	-	-	241031.00 ± 14770.00	220107.00 ± 16752.00	0.351

For non-normally-distributed variables, the Mann-Whitney test was applied and they were described by median and interquartile range (IQR) (Q1-Q3). For normally-distributed variables, the independent samples t-test was used for comparison and they were described by mean ± standard deviation (SD).

^{*}These factors only were checked in patient group

BMI: Body mass index; IMT: Intima-media thickness; TG: Triglyceride; hs-CRP: High-sensitivity C-reactive protein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; FBS: Fasting blood sugar; IL-18: Interleukin-18; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; dsDNA: Double stranded deoxyribonucleic acid

Table 2. Comparison of classified cardiac risk factors and interleukin-18 (IL-18) serum level in patients with systemic lupus erythematosus (SLE)

Variable	Variable level	Percentage	Mean \pm SD	P
BMI (kg/m ²)	Less than 25	65	357 \pm 56	0.450
	More than 25	35	301 \pm 47	
Cholesterol (mg/dl)	Less than 200	30	340 \pm 55	0.960
	More than 200	70	336 \pm 52	
LDL (mg/dl)	Less than 140	75	348 \pm 51	0.797
	More than 140	25	324 \pm 55	
HDL (mg/dl)	Less than 50	23	358 \pm 47	0.339
	More than 50	77	268 \pm 71	
FBS (mg/dl)	Less than 110	5	432 \pm 30	0.070
	More than 110	95	332 \pm 42	
hs-CRP (mg/l)	Less than 3	33	268 \pm 55	0.229
	More than 3	67	373 \pm 54	

For non-normally-distributed variables, the Mann-Whitney test was applied and for normally-distributed variables, the independent samples t-test was used.

BMI: Body mass index; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; FBS: Fasting blood sugar; hs-CRP: High-sensitivity C-reactive protein; SD: Standard deviation

Discussion

The present study showed significantly higher serum levels of IL-18 in patients with SLE compared to the healthy population, indicating the role of IL-18 as an inflammatory marker in patients with SLE.

Recent studies have demonstrated that the plasma level of IL-18 in patients with SLE is significantly higher than that in the healthy population and is associated with the disease activity and its clinical manifestations.^{2,9-15} In our study, the higher levels of serum IL-18 in patients with SLE could be considered as an inflammatory factor. However, even though the disease activity was also higher in higher IL-18 levels, no significant relationship between the level of IL-18 and subclinical atherosclerosis was found.

A systematic review by Jefferis et al.¹⁶ and Teixeira and Tam¹⁷ study reported significantly increased levels of triglyceride (TG) and C-reactive protein (CRP) and decreased levels of high-density lipoprotein (HDL) in patients with higher serum levels of IL-18. Yamagami et al. also reported a significant positive relationship between the IL-18 level and age, BMI, TG, and high-sensitivity CRP (hs-CRP), and a negative correlation with fasting blood sugar (FBS), total cholesterol, and HDL.¹⁸ Tso et al. reported a difference in serum levels of TG between patients with a high and low serum level of IL-18.¹⁰ However, we found no such significant relationships between patients with low and high serum IL-18 levels. It could be because our subjects were in different stages of the disease, therefore, with different types and doses of anti-inflammatory

medications. The lack of correlation between IL-18 and low-density lipoprotein (LDL) in this study can also be due to multiple factors such as differential disease duration, the severity of the disease, and the presence of immunosuppressive treatments.

As reported by Yamagami et al.¹⁸ and Correale et al.,¹⁹ IL-18 has demonstrated a positive correlation with the carotid IMT, as an indicator of systemic atherosclerosis. Other authors have also reported a significant difference between the patients with SLE and the control group in terms of IMT.^{17,20} We also found such a significant difference for the both right lower and left lower carotid artery, where patients had a higher IMT. These findings suggested that IMT could be attributed as a subclinical atherosclerosis indicator in patients with SLE. However, no significant difference in IMT was found between the two groups of patients based on IL-18 level.

Other cytokines may play a role in the increased risk of premature atherosclerosis in patients with SLE. Race and genetics may also influence the inflammatory response leading to plaque formation.^{14,21}

One limitation of this study is to enroll patients after five months of disease. In future works, patients should be evaluated over a longer period.

Conclusion

The serum level of IL-18 was found to be significantly higher in patients with SLE. The level of IL-18 \geq 280 was significantly correlated with the disease activity, but no significant relationship was found between the serum IL-18 and IMT and

subclinical atherosclerosis. Studies with a larger sample size and longer follow-up are recommended.

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Authors' Contribution

ZR: Designing the study, referring the patients, interpretation of data, revising the intellectual content, final revision of the manuscript

MA: Writing the manuscript, gathering data, interpretation of data, revising the comments

NS: Revising the intellectual content, final revision of the manuscript

MS: Author of the primary draft of the manuscript and following up the procedure, analysis of data, final revision of the manuscript

RO: Gathering data, interpretation of data

SS: Gathering data, interpretation of data

HE: Analysis of data, final revision of the manuscript

MRA: Doing Doppler ultrasound and analysis of IMT, interpretation of data

AF: Revising the manuscript, interpretation of data, analysis of data

EA: Analysis of data

Conflict of Interests

Authors have no conflict of interests.

References

- Migliorini P, Anzilotti C, Pratesi F, Quattroni P, Bargagna M, Dinarello CA, et al. Serum and urinary levels of IL-18 and its inhibitor IL-18BP in systemic lupus erythematosus. *Eur Cytokine Netw* 2010; 21(4): 264-71.
- Park JK, Kim JY, Moon JY, Ahn EY, Lee EY, Lee EB, et al. Altered lipoproteins in patients with systemic lupus erythematosus are associated with augmented oxidative stress: A potential role in atherosclerosis. *Arthritis Res Ther* 2016; 18(1): 306.
- Nicolaou O, Kousios A, Hadjisavvas A, Lauwerys B, Sokratous K, Kyriacou K. Biomarkers of systemic lupus erythematosus identified using mass spectrometry-based proteomics: A systematic review. *J Cell Mol Med* 2017; 21(5): 993-1012.
- Jafari-Nakhjavani MR, Abedi-Azar S, Nejati B. Correlation of plasma interleukin-18 concentration and severity of renal involvement and disease activity in systemic lupus erythematosus. *J Nephropathol* 2016; 5(1): 28-33.
- Hulthe J, McPheat W, Samnegard A, Tornvall P, Hamsten A, Eriksson P. Plasma interleukin (IL)-18 concentrations is elevated in patients with previous myocardial infarction and related to severity of coronary atherosclerosis independently of C-reactive protein and IL-6. *Atherosclerosis* 2006; 188(2): 450-4.
- Italiani P, Manca ML, Angelotti F, Melillo D, Pratesi F, Puxeddu I, et al. IL-1 family cytokines and soluble receptors in systemic lupus erythematosus. *Arthritis Res Ther* 2018; 20(1): 27.
- Fauci A, Braunwald E, Kasper D, Hauser S, Longo D, Jameson J, et al. *Harrison's principles of internal medicine*. 17th ed. New York, NY: McGraw Hill; 2008.
- Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35(6): 630-40.
- Zirlik A, Abdullah SM, Gerdes N, MacFarlane L, Schonbeck U, Khera A, et al. Interleukin-18, the metabolic syndrome, and subclinical atherosclerosis: Results from the Dallas Heart Study. *Arterioscler Thromb Vasc Biol* 2007; 27(9): 2043-9.
- Tso TK, Huang WN, Huang HY, Chang CK. Relationship of plasma interleukin-18 concentrations to traditional and non-traditional cardiovascular risk factors in patients with systemic lupus erythematosus. *Rheumatology (Oxford)* 2006; 45(9): 1148-53.
- Bhat OM, Kumar PU, Giridharan NV, Kaul D, Kumar MJ, Dhawan V. Interleukin-18-induced atherosclerosis involves CD36 and NF-kappaB crosstalk in Apo E^{-/-} mice. *J Cardiol* 2015; 66(1): 28-35.
- Duan Z, Zhang Y, Zeng Z, Pan F. Comment on "Inflammasome activation of IL-18 results in endothelial progenitor cell dysfunction in systemic lupus erythematosus". *J Immunol* 2012; 189(2): 499-500.
- Mende R, Vincent FB, Kandane-Rathnayake R, Koelmeyer R, Lin E, Chang J, et al. Analysis of Serum Interleukin (IL)-1beta and IL-18 in Systemic Lupus Erythematosus. *Front Immunol* 2018; 9: 1250.
- Umare V, Pradhan V, Nath S, Rajadhyaksha A, Ghosh K, Nadkarni AH. Impact of functional IL-18 polymorphisms on genetic predisposition and diverse clinical manifestations of the disease in Indian SLE patients. *Lupus* 2019; 28(4): 545-54.
- Xiang M, Feng Y, Wang Y, Wang J, Zhang Z, Liang J, et al. Correlation between circulating interleukin-18 level and systemic lupus

- erythematosus: A meta-analysis. *Sci Rep* 2021; 11(1): 4707.
16. Jefferis BJ, Papacosta O, Owen CG, Wannamethee SG, Humphries SE, Woodward M, et al. Interleukin 18 and coronary heart disease: Prospective study and systematic review. *Atherosclerosis* 2011; 217(1): 227-33.
 17. Teixeira V, Tam LS. Novel insights in systemic lupus erythematosus and atherosclerosis. *Front Med (Lausanne)* 2017; 4: 262.
 18. Yamagami H, Kitagawa K, Hoshi T, Furukado S, Hougaku H, Nagai Y, et al. Associations of serum IL-18 levels with carotid intima-media thickness. *Arterioscler Thromb Vasc Biol* 2005; 25(7): 1458-62.
 19. Correale M, Brunetti ND, Di BM. The pro-inflammatory role of cytokines in the mechanism of atherosclerosis. *G Ital Cardiol (Rome)* 2006; 7(9): 594-603. [In Italian].
 20. Bhatt SP, Handa R, Gulati GS, Sharma S, Pandey RM, Aggarwal P, et al. Atherosclerosis in Asian Indians with systemic lupus erythematosus. *Scand J Rheumatol* 2006; 35(2): 128-32.
 21. Chen S, Jiang F, Ren J, Liu J, Meng W. Association of IL-18 polymorphisms with rheumatoid arthritis and systemic lupus erythematosus in Asian populations: A meta-analysis. *BMC Med Genet* 2012; 13: 107.